I hereby certify that this correspondence is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EV 915685902 US, in an envelope addressed to: Board of Patent Appeals and Interferences, United States Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: October 2, 2006 Signature: (Judy A. Bridgwater)

Docket No.: 381092000720
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of:

Terry P. SNUTCH et al.

Application No.: 09/346,794

Filed: July 2, 1999

For: NOVEL HUMAN CALCIUM CHANNELS AND RELATED PROBES, CELL LINES AND METHODS

Appeal No. 2006-2389

REFERENCE TO RELATED CASE AND VERIFICATION OF STATUS

Board of Patent Appeals and Interferences
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

An oral hearing is scheduled in this Appeal on 19 October 2006 at 9:00 a.m. Applicants appreciate the telephonic confirmation that their Confirmation of Appearance mailed 20 September 2006 will be accepted in view of the non-receipt by Applicants' representative of the Notice of Hearing mailed 28 August 2006. The Notice was obtained due to a routine check of PAIR and the mailed copy has never been received by the undersigned.

At the time the Appeal Brief was filed in this Application on 13 September 2005, there were no related appeals or interferences that would be germane to the present case. However, since then,

co-pending Application 09/611,257 was appealed on 3 March 2006, along with a Pre-Appeal Brief Request for Review. The Request for Review resulted in remanding the case to the Examiner who withdrew rejections under 35 U.S.C. 101 and 35 U.S.C. 112, similar to those at issue in the present Application. Copies of the Notice of Appeal, Request for Review, Notice of Panel Decision, subsequent Office Action and Response thereto, and Notice of Allowance are enclosed for the convenience of the Board.

The claims in the present case are directed to a method to identify agonists and antagonists of T-type calcium channels encoded by nucleic acids similar to those represented by SEQ ID NO. 23 of the present claims. In general, the position of the Examiner in the present application is that the resulting agonists and antagonists would not be useful.

In the related '257 Application, which is a continuation-in-part of the present case, claims are directed to an isolated DNA molecule which will provide a suitable T-type calcium channel sub-unit for use in such assays where the amino acid sequence of the sub-unit is SEQ ID NO. 24 or SEQ ID NO. 37. SEQ ID NO.24 is the amino acid sequence encoded by SEQ ID NO. 23, SEQ ID NO. 37 represents a human counterpart.

As the Board will note, the withdrawn rejections in the related '257 case were made on substantially the same basis as those at issue in the present application. Therefore, it is believed that the prosecution in the '257 case is relevant to the issues herein.

One last housekeeping item: Applicants appreciate that the Amendment After Final submitted in this case on 26 January 2006 has been entered and the claims at issue are now claims 28-31, 37 and 40. An extra copy of these claims is submitted for the convenience of the

Board; Applicants greatly appreciate the entry of this Amendment as indicated in the Advisory Action mailed 20 April 2006.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 381092000720.

Dated: October 2, 2006

Respectfully submitted,

By Kate H. Murashige

Kate H. Murashige
Registration No.: 29,959
MORRISON & FOERSTER LLP
12531 High Bluff Drive
Suite 100
San Diego, California 92130-2040
(858) 720-5112

COPY

Atty Docket No.: 381092000721

Inventor: Terrance P. SNUTCH et al.

Application No.: 09/611,257 **Filing Date:** July 6, 2000
Title: MAMMALIAN T-TYPE CALCIUM CHANNELS

Documents Filed:

Transmittal (1 page)

Fee Transmittal (1 page + duplicate for fee processing)

Two Month Request for Extension of Time Under 37 CFR 1.136(a) (1 page)

Notice of Appeal (1 page)

Pre-Appeal Brief Request for Review (1 page)

Reasons Review is Requested (3 pages)

EV761644405US

Via: Express Mail: Airbill No. EV 761644405 US
Sender's Initials: KHM1/mlc3

Date: March 3, 2006

Atty Docket No.: 381092000721

Inventor: Terrance P. SNUTCH et al.

Application No.: 09/611,257 **Filing Date:** July 6, 2000
Title: MAMMALIAN T-TYPE CALCIUM CHANNELS

Documents Filed:

Transmittal (1 page)

Fee Transmittal (1 page + duplicate for fee processing)

Two Month Request for Extension of Time Under 37 CFR 1.136(a) (1 page)

Notice of Appeal (1 page)

Pre-Appeal Brief Request for Review (1 page)

Reasons Review is Requested (3 pages)

Via: Express Mail: Airbill No. EV 761644405 US
Sender's Initials: KHM1/mlc3

Date: March 3, 2006

BLM

MAR 10 2006

MORRISON & FOERSTER
SAN DIEGO

OIPE IAP58

MAR 03 2006

PATENT & TRADEMARK OFFICE

23

PTO/SB/21 (09-04)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	09/611,257
Filing Date	July 6, 2000
First Named Inventor	Terrance P. SNUTCH
Art Unit	1649
Examiner Name	D. KOLKER
Attorney Docket Number	381092000721

Total Number of Pages in This Submission	9

ENCLOSURES (*Check all that apply*)

- [X] Fee Transmittal Form (1 page + duplicate for fee processing)
- [ ] Fee Attached
- [ ] Amendment/Reply
- [ ] After Final
- [ ] Affidavits/declaration(s)
- [X] Extension of Time Request (1 page)
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
- [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
- [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [X] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief) (1 page)**
- [ ] Proprietary Information
- [ ] Status Letter
- [X] Other Enclosure(s) (please Identify below):

Pre-Appeal Brief Request for review (1 page)
Reasons Review is Requested (3 pages)
Return Receipt Postcard

Remarks

Customer No. 25225

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	MORRISON & FOERSTER LLP		
Signature	Kate H. Murashige		
Printed name	Kate H. Murashige		
Date	March 3, 2006	Reg. No.	29,959

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EV 761644405 US, in an envelope addressed to: MS AF, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: March 3, 2006 Signature: Marian L. Christopher (Marian L. Christopher)

sd-304761

PTO/SB/17 (12-04v2)
Approved for use through 7/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no person are required to respond to a collection of information unless it displays a valid OMB control number.

Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2005

[X] Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT	($) 475.00

Complete if Known	
Application Number	09/611,257
Filing Date	July 6, 2000
First Named Inventor	Terrance P. SNUTCH
Examiner Name	D. KOLKER
Art Unit	1649
Attorney Docket No.	381092000721

METHOD OF PAYMENT (check all that apply)

[ ] Check [ ] Credit Card [ ] Money Order [ ] None [ ] Other (please identify): _____

[X] Deposit Account Deposit Account Number: 03-1952 Deposit Account Name: Morrison & Foerster LLP

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

[X] Charge fee(s) indicated below
[ ] Charge fee(s) indicated below, **except for the filing fee**
[X] Charge any additional fee(s) or underpayment of fee(s) under 37 CFR 1.16 and 1.17
[X] Credit any overpayments

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
Application Type	Fee ($)	Small Entity Fee ($)	Fee ($)	Small Entity Fee ($)	Fee ($)	Small Entity Fee ($)	Fees Paid ($)
Utility	300	150	500	250	200	100	0.00
Design	200	100	100	50	130	65	0.00
Plant	200	100	300	150	160	80	0.00
Reissue	300	150	500	250	600	300	0.00
Provisional	200	100	0	0	0	0	0.00

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims		Extra Claims	Fee ($)		Fee Paid ($)
_____	- =	_____	x _____	=	0.00

Indep. Claims		Extra Claims	Fee ($)		Fee Paid ($)
_____	- =	_____	x _____	=	0.00

Multiple Dependent Claims	
Fee ($)	Fee Paid ($)
_____	0.00

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets		Extra Sheets		Number of each additional 50 or fraction thereof		Fee ($)		Fee Paid ($)
_____	- 100 =	_____	/50	_____	(round up to a whole number) x	_____	=	_____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other (e.g., late filing surcharge): 2252 Extension for response within second month	225.00
2401 Notice of appeal	250.00

SUBMITTED BY

Signature	Kate H. Murashige	Registration No. (Attorney/Agent) 29,959	Telephone (858) 720-5112
Name (Print/Type)	Kate H. Murashige		Date March 3, 2006

sd-304762

Duplicate Copy
For Fee Processing

PTO/SB/17 (12-04v2)
Approved for use through 7/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no person are required to respond to a collection of information unless it displays a valid OMB control number.

Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2005

[X] Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 475.00

Complete if Known	
Application Number	09/611,257
Filing Date	July 6, 2000
First Named Inventor	Terrance P. SNUTCH
Examiner Name	D. KOLKER
Art Unit	1649
Attorney Docket No.	381092000721

METHOD OF PAYMENT (check all that apply)

[ ] Check [ ] Credit Card [ ] Money Order [ ] None [ ] Other (please identify): _____

[X] Deposit Account Deposit Account Number: 03-1952 Deposit Account Name: Morrison & Foerster LLP

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

[X] Charge fee(s) indicated below
[ ] Charge fee(s) indicated below, except for the filing fee
[X] Charge any additional fee(s) or underpayment of fee(s) under 37 CFR 1.16 and 1.17
[X] Credit any overpayments

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
Application Type	Fee ($)	Small Entity Fee ($)	Fee ($)	Small Entity Fee ($)	Fee ($)	Small Entity Fee ($)	Fees Paid ($)
Utility	300	150	500	250	200	100	0.00
Design	200	100	100	50	130	65	0.00
Plant	200	100	300	150	160	80	0.00
Reissue	300	150	500	250	600	300	0.00
Provisional	200	100	0	0	0	0	0.00

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims	Extra Claims	Fee ($)	Fee Paid ($)
_____ - =	_____ x	_____ =	0.00

Indep. Claims	Extra Claims	Fee ($)	Fee Paid ($)
_____ - =	_____ x	_____ =	0.00

Multiple Dependent Claims	
Fee ($)	Fee Paid ($)
_____	0.00

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee ($)	Fee Paid ($)
_____ - 100 =	_____ /50	_____ (round up to a whole number) x	_____ =	_____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other (e.g., late filing surcharge): 2252 Extension for response within second month	225.00
2401 Notice of appeal	250.00

SUBMITTED BY

Signature	Kate H. Murashige	Registration No. (Attorney/Agent)	29,959	Telephone	(858) 720-5112
Name (Print/Type)	Kate H. Murashige			Date	March 3, 2006

sd-304762

PTO/SB/22 (12-04)
Approved for use through 7/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless if displays a valid OMB control number.

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) FY 2005 (Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)	Docket Number (Optional) 381092000721
Application Number 09/611,257	Filed July 6, 2000
For MAMMALIAN T-TYPE CALCIUM CHANNELS	
Art Unit 1649	Examiner D. KOLKER

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	
☐ One month (37 CFR 1.17(a)(1))	$120	$60	$
☒ Two months (37 CFR 1.17(a)(2))	$450	$225	$ 225.00
☐ Three months (37 CFR 1.17(a)(3))	$1020	$510	$
☐ Four months (37 CFR 1.17(a)(4))	$1590	$795	$
☐ Five months (37 CFR 1.17(a)(5))	$2160	$1080	$

☒ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check in the amount of the fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 03-1952 ~~I have enclosed a duplicate copy of this sheet~~. Fee Transmittal form (PTO/SB/17) is attached to this submission in duplicate.

I am the

☐ applicant/inventor.

☐ assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96).

☒ attorney or agent of record. Registration Number 29,959

☐ attorney or agent under 37 CFR 1.34. Registration number if acting under 37 CFR 1.34 _____

Kate H. Murashige
Signature

March 3, 2006
Date

Kate H. Murashige
Typed or printed name

(858) 720-5112
Telephone Number

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

☒ Total of 1 forms are submitted.

sd-304758

PTO/SB/31 (04-05)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number

NOTICE OF APPEAL FROM THE EXAMINER TO THE BOARD OF PATENT APPEALS AND INTERFERENCES	Docket Number (Optional) 381092000721

In re Application of Terrance P. SNUTCH et al.	
Application Number 09/611,257	Filed July 6, 2000
For MAMMALIAN T-TYPE CALCIUM CHANNELS	
Art Unit 1649	Examiner D. KOLKER

Applicant hereby **appeals** to the Board of Patent Appeals and Interferences from the last decision of the examiner.

The fee for this Notice of Appeal is (37 CFR 41.20(b)(1)) $ 500.00

[X] Applicant claims small entity status. See 37 CFR 1.27. Therefore, the fee shown above is reduced by half, and the resulting fee is: $ 250.00

[ ] A check in the amount of the fee is enclosed.

[ ] Payment by credit card. Form PTO-2038 is attached.

[ ] The Director has already been authorized to charge fees in this application to a Deposit Account. I have enclosed a duplicate copy of this sheet.

[X] The Director is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 03-1952 ~~I have enclosed a duplicate copy of this sheet.~~ Fee Transmittal form (PTO/SB/17) is attached to this submission in duplicate.

[X] A petition for an extension of time under 37 CFR 1.136(a) (PTO/SB/22) is enclosed.

I am the

[ ] applicant /inventor.

[ ] assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)

[X] attorney or agent of record.
Registration number 29,959

[ ] attorney or agent acting under 37 CFR 1.34.
Registration number if acting under 37 CFR 1.34. _____

Kate H. Murashige
Signature

Kate H. Murashige
Typed or printed name

(858) 720-5112
Telephone number

March 3, 2006
Date

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

[X] *Total of 1 forms are submitted.

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EV 761644405 US, in an envelope addressed to: MS AF, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: March 3, 2006 Signature: Marian L. Christopher (Marian L. Christopher)

sd-304759

COPY

Doc Code: AP.PRE.REQ

PTO/SB/33 (07-05)
Approved for use through xx/xx/200x. OMB 0651-00xx
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PRE-APPEAL BRIEF REQUEST FOR REVIEW	Docket Number (Optional) 381092000721

Application Number	Filed
09/611,257	July 6, 2000

First Named Inventor: Terrance P. SNUTCH et al.

Art Unit	Examiner
1649	D. KOLKER

Applicant requests review of the final rejection in the above-identified application. No amendments are being filed with this request.

This request is being filed with a notice of appeal.

The review is requested for the reason(s) stated on the attached sheet(s).
Note: No more than five (5) pages may be provided.

I am the

- [ ] applicant /inventor.
- [ ] assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)
- [x] attorney or agent of record. Registration number 29,959
- [ ] attorney or agent acting under 37 CFR 1.34. Registration number if acting under 37 CFR 1.34. _____

Kate H. Murashige
Signature

Kate H. Murashige
Typed or printed name

(858) 720-5112
Telephone number

March 3, 2006
Date

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

[X] *Total of 1 forms are submitted.

sd-304760

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EV 761644405 US, in an envelope addressed to: MS AF, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: March 3, 2006 Signature: (Marian L. Christopher)

Docket No.: 381092000721
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Terrance P. SNUTCH et al.

Application No.: 09/611,257 Confirmation No.: 5449

Filed: July 6, 2000 Art Unit: 1649

For: MAMMALIAN T-TYPE CALCIUM CHANNELS Examiner: D. KOLKER

REASONS REVIEW IS REQUESTED

MS AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Applicants request review of two matters: First, the Examiner's refusal to consider published documents submitted in support of applicants' position that the claimed subject matter does not lack utility and citation of MPEP 609.04(b)(III) are inappropriate. This is true especially in view of the assertion that a PubMed search conducted by the Examiner failed to reveal entries relevant to this issue which was contained in the Final Rejection to which the relevant response was filed. Second, the basis for finding lack of utility, is and has been, throughout the prosecution, inconsistent with the Patent Office's own practices and guidelines and ignores documentation already of record.

A. Evidence of Record

Regardless of the refusal of the Examiner to consider the documents listed on page 9 of the Response to Final Rejection, U.S. 6,309,858 must be considered as it is already of record. It was submitted on a Supplementary Information Disclosure Statement on 20 September 2004. An additional document cited in support of Applicants' position, U.S. 6,358,706 is clearly of record as it was made the basis for an art rejection. Either of these documents would be sufficient to support applicants claim that the invention is useful, even if the publications listed on page 9 of the Response to Final Rejection were not considered.

B. Evidence for which Consideration was Refused

As to these documents, they are directly in response to the assertion by the Examiner in the Final Rejection that "For example, a PubMed search conducted 27 September 2005 for 't-type calcium channel schizophrenia' revealed no entries through 2000... and a search for 't-type calcium channel Parkinson's' revealed no entries through 2000...". Contrary to the implication of the Examiner's reference to MPEP 609.04(b)(III) relating to requirements for filing an Information Disclosure Statement after final rejection, Applicants do not believe submission of an IDS is an appropriate form of submission. The IDS submission is designed to fulfill Applicants' duty under 37 CFR 1.56 to disclose information material to patentability which is defined as information that "establishes, by itself or in combination with other information, a *prima facie* case of unpatentability of a claim"; or "*refutes or is inconsistent with* a position that the Applicant takes in (i) opposing an argument of unpatentability relied on by the Office or (ii) asserting an argument of patentability." The information submitted is neither relevant to a *prima facie* case of unpatentability nor is it inconsistent with Applicants' position.

Applicants believe that the Examiner has unduly prolonged prosecution by refusing to consider these documents. As the Office is concerned by the number of continuations and RCEs, this might be considered an example of one of the causes.

C. The Examiner's Position on Utility is Inconsistent with The Practice of the Office and with Reality.

As has been noted throughout the prosecution, many parties have endeavored to obtain recombinant types of T-type ion channels because these channels are implicated in a multiplicity of undesirable conditions, including those set forth in the specification. Applicants have presented experimental evidence in the application itself that the claimed recombinant DNA molecule does encode a T-type ion channel. Further, regardless of the additional publications which the Examiner has refused to consider, the two cited patents which are clearly of record demonstrate that prior to the filing of the present application, the association of T-type ion channels with the specified conditions was understood in the art. (The Office appeared to recognize this in issuing these patents0but, again Applicants emphasize they are not arguing a precedential value for these patents, but only their relevance as prior art). The publications submitted on page 9 of the Response to Final Rejection are merely cumulative to the evidence set forth in these patents, and have been characterized as such. Applicants believe the patents of record are adequate to demonstrate utility.

It would be appreciated if the Appeals Conference Committee would review in detail the Response to Final Rejection, which Applicants believe establishes patentability of the claimed subject matter.

Conclusion:

No *prima facie* case of lack of utility has been made out during the prosecution, and Applicants request that the rejection be reconsidered and the application be passed to issue.

Dated: March 3, 2006

Respectfully submitted,

By _____

Kate H. Murashige
Registration No.: 29,959
MORRISON & FOERSTER LLP
12531 High Bluff Drive
Suite 100
San Diego, California 92130-2040
Telephone: (858) 720-5112
Facsimile: (858) 720-5125

UNITED STATES PATENT AND TRADEMARK OFFICE

COPY

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/611,257	07/06/2000	Terrance P. Snutch	381092000721	5449

25225 7590 05/15/2006

KHM

MORRISON & FOERSTER LLP
12531 HIGH BLUFF DRIVE
SUITE 100
SAN DIEGO, CA 92130-2040

RECEIVED

MAY 19 2006

MORRISON & FOERSTER LLP
SAN DIEGO

EXAMINER	
KOLKER, DANIEL E	
ART UNIT	PAPER NUMBER
1649	

DATE MAILED: 05/15/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

No Docketing Required
Reviewed by Docketing
Initials

Application Number	Application/Control No.	Applicant(s)/Patent under Reexamination	
	09/611,257	SNUTCH ET AL.	
	Daniel Kolker	Art Unit 1649	

Document Code - AP.PRE.DEC

Notice of Panel Decision from Pre-Appeal Brief Review

This is in response to the Pre-Appeal Brief Request for Review filed 3/03/06.

1. ☐ **Improper Request** – The Request is improper and a conference will not be held for the following reason(s):

☐ The Notice of Appeal has not been filed concurrent with the Pre-Appeal Brief Request.
☐ The request does not include reasons why a review is appropriate.
☐ A proposed amendment is included with the Pre-Appeal Brief request.
☐ Other: .

The time period for filing a response continues to run from the receipt date of the Notice of Appeal or from the mail date of the last Office communication, if no Notice of Appeal has been received.

2. ☐ **Proceed to Board of Patent Appeals and Interferences** – A Pre-Appeal Brief conference has been held. The application remains under appeal because there is at least one actual issue for appeal. Applicant is required to submit an appeal brief in accordance with 37 CFR 41.37. The time period for filing an appeal brief will be reset to be one month from mailing this decision, or the balance of the two-month time period running from the receipt of the notice of appeal, whichever is greater. Further, the time period for filing of the appeal brief is extendible under 37 CFR 1.136 based upon the mail date of this decision or the receipt date of the notice of appeal, as applicable.

☐ The panel has determined the status of the claim(s) is as follows:
Claim(s) allowed: _____.
Claim(s) objected to: _____.
Claim(s) rejected: _____.
Claim(s) withdrawn from consideration: _____.

3. ☐ **Allowable application** – A conference has been held. The rejection is withdrawn and a Notice of Allowance will be mailed. Prosecution on the merits remains closed. No further action is required by applicant at this time.

4. ☒ **Reopen Prosecution** – A conference has been held. The rejection is withdrawn and a new Office action will be mailed. No further action is required by applicant at this time.

All participants:

(1) *Janet L. Andres*. JLA

(2) *Daniel Kolker*. DK

(3) *Sharon Turner*. ST

(4)_____.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

KHM

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/611,257	07/06/2000	Terrance P. Snutch	381092000721	5449

25225 7590 04/14/2006

MORRISON & FOERSTER LLP
12531 HIGH BLUFF DRIVE
SUITE 100
SAN DIEGO, CA 92130-2040

EXAMINER	
KOLKER, DANIEL E	

ART UNIT	PAPER NUMBER
1649	

DATE MAILED: 04/14/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

MR DOCKETED: 4-OA RESP
DUE DATE: 7/14/2006
FINAL DUE DATE: 10/14/2006

Office Action Summary	Application No.	Applicant(s)	
	09/611,257	SNUTCH ET AL.	
	Examiner	Art Unit	
	Daniel Kolker	1649	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *03 March 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,2,4-6,14 and 18-26* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,2,4-6 and 19-26* is/are rejected.

7)☒ Claim(s) *18* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's request for reconsideration of the finality of the rejection of the last Office action is persuasive and, therefore, the finality of that action is withdrawn.

2. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

3. Claims 1 – 2, 4 – 6, 14, and 18 – 26 are pending and under examination.

Withdrawn Rejections

4. The rejections set forth under 35 USC §§ 101 and 112 are withdrawn. The claimed nucleic acids are asserted to encode alpha G1 subunits of T-type calcium channels. SEQ ID NO:24 is the rat calcium channel subunit (specification, paragraph spanning pp. 11 – 12). SEQ ID NO:37 is the human sequence (see specification p. 22, note amendment to specification filed 15 September 2004). The specification asserts that the claimed channels are useful for screening for drugs for treatment of many diseases, including epilepsy (see specification, p. 9 line 24).

The prior art recognized importance of T-type channels in epilepsy. See for example Lee et al. (1999. Journal of Neuroscience 19:1912-1921, particularly final paragraph on p. 1920). Coulter et al. (1989. Annals of Neurology 25:582-593) teach that the anti-convulsant drugs ethosuximide and dimethadione both decrease activity of T-type calcium channels. The specification discloses that the proteins encoded by the claimed nucleic acids pass calcium in a manner that is consistent with their being T-type calcium channels. See for example specification pp. 20 and 23 as well as Figures 3, 4, and 7. Thus the data presented in the specification support the assertion that the claimed nucleic acids encode T-type calcium channels.

The proteins of SEQ ID NO:24 and 37 are 91% identical to each other and are similar to other prior art proteins. U.S. Patent 6,358,706 (filed 22 October 1999, issued 19 March 2002) discloses SEQ ID NO:5, which is 91% identical to instant SEQ ID NO:24 and 99% identical to instant SEQ ID NO:37. The '706 patent discloses that T-type calcium channels have "important consequences for epilepsy" and the patent states that modulators of the channels found in screening assays can be candidates for treatment of epilepsy (see column 17). Additionally the calcium currents passed by these channels are sensitive to the known anti-convulsant ethosuximide (see column 27 line 55). Perez-Reyes et al. (1998 Nature 391:896 – 906, of

record) teach a rat alpha 1G subunit with the sequence deposited as GenBank accession number AF027984. The sequence is 99.8% identical to instant SEQ ID NO:24 and is 91.9% identical to instant SEQ ID NO:37. The reference teaches that alpha 1A calcium channels, when mutated, lead to epileptic phenotypes (see p. 896 second column).

For the reasons set forth above, the asserted utility for the claimed invention, namely using the nucleic acids in screening assays to find candidates for treatment of epilepsy, constitutes a specific and substantial credible utility. Thus the rejection is withdrawn.

New rejections and Objections

Claim Objections

5. Claim 14 is objected to because of the following informalities: it has a typographical error. It reads "n isolated" but should read "An isolated". Appropriate correction is required.

Claim Rejections - 35 USC § 101

6. Claims 1 – 2, 4 – 6, and 19 – 25 rejected under 35 U.S.C. 101 because the claimed invention is directed to non-statutory subject matter.

Claim is drawn to a recombinant DNA molecule which comprises nucleic acid sequences encoding either SEQ ID NO:24 or 37. The claim requires that said sequence be operably linked to control sequences to effect its expression. Genomic DNA molecules residing within an animal are operably linked to control sequences called promoters and enhancers. The recitation of "recombinant" is a product-by-process limitation which does not distinguish the claimed invention over the naturally occurring products. Amendment of claim 1 to recite "An isolated DNA" is recommended.

Claim Rejections - 35 USC § 102

7. Applicant is reminded that the effective filing date for all pending claims is 6 July 2000 for the reasons set forth in the office action mailed 21 December 2004. Applicant did not traverse the examiner's conclusion of the effective filing date.

8. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

Claims 1, 4 – 6, 14, and 19 – 26 are rejected under 35 U.S.C. 102(a) as being anticipated by McRory et al. (1999. Society for Neuroscience Abstracts 25(1-2):197. Presented at 29th Annual Meeting of the Society for Neuroscience October 23 – 28 1999).

The claims encompass isolated nucleic acids, recombinant DNA molecules, cells, and methods of effecting production wherein the nucleic acid encodes SEQ ID NO:24. The specification discloses that SEQ ID NO:24 is rat calcium channel alpha 1G subunit (specification, paragraph spanning pp. 11 – 12). McRory teaches isolation and characterization of nucleic acids encoding several rat calcium channel subunits, including alpha 1G. Furthermore the reference teaches expressing the nucleic acids in HEK 293 cells, which are mammalian cells. The expression is sufficient to effect production of the protein, as the reference teaches that it allows the determination that the nucleic acid encodes a functional alpha 1G subunit. While the reference is silent with respect to the sequence of the rat nucleic acid and protein, the products disclosed in the reference are indistinguishable from the claimed invention.

Conclusion

9. Claims 1 – 2, 4 – 6, 14, and 19 – 26 are rejected.

Claim 18 is objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

10. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Daniel Kolker whose telephone number is (571) 272-3181. The examiner can normally be reached on Mon - Fri 8:30AM - 5:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Janet Andres can be reached on (571) 272-0867. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Daniel E. Kolker, Ph.D.
April 7, 2006

JANET L. ANDRES
SUPERVISORY PATENT EXAMINER

Notice of References Cited	Application/Control No. 09/611,257	Applicant(s)/Patent Under Reexamination SNUTCH ET AL.	
	Examiner Daniel Kolker	Art Unit 1649	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-6,358,706	03-2002	Dubin et al.	435/69.1
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Lee et al. 1999. Journal of Neuroscience 19:1912-1921
	V	Coulter et al. 1989. Annals of Neurology 25:582-593
	W	McRory et al. 1999. Society for Neuroscience Abstracts 25(1-2):197
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

The Journal of Neuroscience, March 15, 1999, 19(6):1912–1921

Cloning and Expression of a Novel Member of the Low Voltage-Activated T-Type Calcium Channel Family

Jung-Ha Lee,[1] Asif N. Daud,[1] Leanne L. Cribbs,[1] Antonio E. Lacerda,[2] Alexei Pereverzev,[3] Udo Klöckner,[4] Toni Schneider,[3] and Edward Perez-Reyes[1]

[1]Department of Physiology, Loyola University Medical Center, Maywood, Illinois 60153, [2]Rammelkamp Center for Research and Education, MetroHealth Medical Center, Cleveland, Ohio 44109, and Departments of [3]Physiology and [4]Vegetative Physiology, University of Cologne, D50931 Cologne, Germany

Low voltage-activated Ca^{2+} channels play important roles in pacing neuronal firing and producing network oscillations, such as those that occur during sleep and epilepsy. Here we describe the cloning and expression of the third member of the T-type family, $\alpha 1I$ or $Ca_vT.3$, from rat brain. Northern analysis indicated that it is predominantly expressed in brain. Expression of the cloned channel in either *Xenopus* oocytes or stably transfected human embryonic kidney-293 cells revealed novel gating properties. We compared these electrophysiological properties to those of the cloned T-type channels $\alpha 1G$ and $\alpha 1H$ and to the high voltage-activated channels formed by $\alpha 1E\beta_3$. The $\alpha 1I$ channels opened after small depolarizations of the membrane similar to $\alpha 1G$ and $\alpha 1H$ but at more depolarized potentials. The kinetics of activation and inactivation were dramatically slower, which allows the channel to act as a Ca^{2+} injector. In oocytes, the kinetics were even slower, suggesting that components of the expression system modulate its gating properties. Steady-state inactivation occurred at higher potentials than any of the other T channels, endowing the channel with a substantial window current. The $\alpha 1I$ channel could still be classified as T-type by virtue of its criss-crossing kinetics, its slow deactivation (tail current), and its small (11 pS) conductance in 110 mM Ba^{2+} solutions. Based on its brain distribution and novel gating properties, we suggest that $\alpha 1I$ plays important roles in determining the electroresponsiveness of neurons, and hence, may be a novel drug target.

Key words: molecular cloning; calcium channel; CNS; thalamus; anticonvulsant; epilepsy

Voltage-gated calcium channels can be subdivided into two classes based on the voltage required to trigger channel opening. Low voltage-activated (LVA) Ca^{2+} channels begin to open after small depolarizations (10 mV) of the plasma membrane, whereas high voltage-activated (HVA) channels require much stronger depolarizations (40 mV). Most voltage-gated Na^+ channels open somewhere between these two extremes. Entry of Ca^{2+} ions causes membrane depolarization. LVA Ca^{2+} channels activate at potentials low enough to gate the activity of other depolarizing voltage-activated ion channels. This led to the hypothesis that LVA channels could act as pacemaker currents, controlling the activity of other voltage-gated ion channels. A clear example of this phenomenon is the thalamic low-threshold Ca^{2+} spike that is crowned with a burst of action potentials mediated by Na^+ channels (Llinas and Jahnsen, 1982). Patch-clamp recordings demonstrated that T-type Ca^{2+} channels mediated the low-threshold spike and that they are involved in rebound burst firing, oscillations, and resonance (for review, see Huguenard, 1996).

Molecular cloning of ion channels has revealed a greater diversity than was expected from electrophysiological studies of endogenous currents. For HVA Ca^{2+} channels, there are at least seven genes encoding $\alpha 1$ subunits, four for β, two for γ, and one for $\alpha 2$ (Bech-Hansen et al., 1998; Letts et al., 1998; Ophoff et al., 1998; Strom et al., 1998). Expression of these $\alpha 1$ subunits led to the induction of typical HVA currents in terms of their biophysical and pharmacological properties. Along with these expected properties, some HVA channels exhibited properties that were once considered specific to T-type channels. Specifically, fast inactivation, inactivation at negative membrane potentials, and block by micromolar concentrations of nickel are no longer distinguishing features (Ellinor et al., 1993; Soong et al., 1993; Zamponi et al., 1996). However, T-type currents can still be distinguished from HVA currents by the following criteria: low voltage-activation, criss-crossing pattern of currents, slow deactivation, and tiny single-channel conductance (Matteson and Armstrong, 1986; Carbone and Lux, 1987; Fox et al., 1987; Randall and Tsien, 1997).

Recently our lab has published the cloning and expression of two new $\alpha 1$ subunits, $\alpha 1G$ and $\alpha 1H$, that display all the characteristic features of T-type currents (Cribbs et al., 1998; Perez-Reyes et al., 1998). In this study we report the cloning of a third member of this T-type channel family, $\alpha 1I$. We compared its electrophysiological properties to those of the cloned T-type channels $\alpha 1G$ and $\alpha 1H$ and to the high voltage-activated channels formed by $\alpha 1E\beta_3$. Based on its brain distribution and novel gating properties, we suggest that $\alpha 1I$ plays important roles in determining the electroresponsiveness of neurons.

MATERIALS AND METHODS

cDNA library screening. A rat brain λgt10 cDNA library (catalog #RL3005a; Clontech, Palo Alto, CA) was screened using conventional filter hybridization according to the manufacturer's protocol. All cDNA probes were released from the vector by restriction digestion, separated on agarose gels, and purified using the Qiaquick gel extraction kit (Qiagen, Valencia, CA). Probes were labeled using ^{32}P-α-dCTP and the

Received Oct. 7, 1998; revised Dec. 1, 1998; accepted Dec. 23, 1998.

This work was supported by a grant from the National Institutes of Health to E.P.-R. (HL57828). We thank Qun Jiang for technical assistance.

Correspondence should be addressed to Edward Perez-Reyes, Department of Physiology, Loyola University Medical Center, 2160 South First Avenue, Maywood, IL 60153.

RadPrime DNA labeling system (Life Technologies, Grand Island, NY). Probes were derived from either Integrated Molecular Analysis of Genomes and their Expression (IMAGE) Consortium (LLNL) clones (Lennon et al., 1996) (ID numbers 402278 and 50902; obtained from Genome Systems, St. Louis. MO) or PCR products. PCR primers were designed from either partial clones (IIS1f, N45) or from the genomic clone 206C7 (GenBank accession number AL008716; direct submission by J. Burgess, Wellcome Trust Genome Campus, Cambridgeshire, UK). The PCR primer sequences were as follows: IS1f, TGC ACG TGG TTT GA(AG) TG(TC) GT; IIS2r, GGC CAG CTT CAG (GAT)AT CAT (CT)TC; IIS1f, ATG GCT ATC CTG GTG AAC AC; IIIS1r, TGG GCA ATG ATG GT(CT) TG(AG) CA; IIIS1f, TTC CGG GTC CTG TG(TC) CA(AG) AC; and N45, GAT GAT GGT GGG (AG)TT GAT. The primers are named according to their approximate location in the protein and their direction (f, forward; r, reverse). The full-length construct was assembled from five cDNA clones (Fig. 1*A*) in the vector pGEM-HEA (a modified version of pGEM-HE; Liman et al., 1992; gift from Kenton Swartz, National Institutes of Health, Bethesda, MD). This vector contains 5′ and 3′ untranslated regions from a *Xenopus* β globin gene. Because of poor growth of bacterial cultures (INVαF′; Invitrogen, Carlsbad, CA) transformed with this construct, we recloned the full-length cDNA into pSP73 (Promega, Madison, WI) along with the 5′ globin sequence [vector coordinates, *Kpn*I (26)/*Xma*I (89)]. The same full-length cDNA was also subcloned into pcDNA3 (Invitrogen) for expression in mammalian cells. The sequence of α1I was determined on both strands of the plasmid using oligonucleotide primers, Sequenase 2.0 (Amersham, Arlington Heights, IL), a digitizer, and WDNASIS software (Hitachi, San Bruno, CA). Regions of compressed sequence were resolved using the 7-deaza-GTP sequencing reaction mix (Amersham).

Northern analysis. Northern blots of 2 μg of mRNA were obtained from either Origene (Rockville, MD) or Clontech. The blots were hybridized at 42°C for 16–20 hr in standard solutions (Sambrook et al., 1989) containing 50% formamide. Blots were washed up to 65°C in a final buffer of 0.1× SSC (15 mM NaCl and 1.5 mM Na citrate) and 0.1% SDS, then exposed to x-ray film (Hyperfilm MP; Amersham) at −80°C between two intensifying screens. The probe was an *Nco*I fragment (nucleotides 5142–6197) of clone ME4, which includes the last 363 bp of the coding region and 692 bp of 3′ untranslated region; none of this sequence is found in either α1G or α1H.

Oocyte expression. Capped cRNA was synthesized from plasmid linearized with *Eco*RI using T7 RNA polymerase (Ambion, Austin, TX). The concentration of cRNA was measured spectrophotometrically. Oocytes were prepared from *Xenopus laevis* (Xenopus One, Ann Arbor, MI) using standard techniques (Leonard and Snutch, 1991). Each oocyte was injected with 2–10 ng of cRNA in a volume of 50 nl. The results were obtained from five batches of oocytes derived from five frogs.

Electrophysiological analysis of injected oocytes. Oocytes were voltage-clamped using a two-microelectrode voltage-clamp amplifier (model OC-725B; Warner Instrument, Hampden, CT). The standard bath solution contained the following (in mM): 10 $Ba(OH)_2$, 90 NaOH, 1 KOH, 0.1 EDTA, and 5 HEPES, adjusted to pH 7.4 with methanesulfonic acid. Voltage and current electrodes (1.5–1.8 MΩ tip resistance) were filled with 3 M KCl. Except where noted, data were filtered at 1 kHz (model 902 filter; Frequency Devices, Haverhill, MA) and digitized at 4 kHz using the pClamp system (Digidata 1200 and pClamp 6.0; Axon Instruments, Foster City, CA). In some experiments, oocytes were injected with 50 nl of 25 mM BAPTA (Molecular Probes, Eugene, OR). Oocytes were allowed to recuperate for at least 1 hr but not more than four.

For single-channel recording, the vitelline membrane was removed with forceps after shrinking in hypertonic media (120 mM K^+-aspartate, 25 mM KCl, 1 mM $MgCl_2$, 10 M EGTA, and 10 mM HEPES, pH 7.4) (Methfessel et al., 1986). Oocytes were then transferred to a depolarizing bath solution containing (in mM): 120 K^+-glutamate, 25 KCl, 1 Ca^{2+}-ATP, 2 EGTA, 10 glucose, and 10 HEPES, pH 7.4 (Lacerda et al., 1994). Pipettes were made from 7052 glass tubing, and the tips were coated with Sylgard 184 (Dow Corning, Midland, MI). The pipette solution contained (in mM): 115 $BaCl_2$, 1 EGTA, and 10 HEPES, pH 7.4 (Lacerda et al., 1994). Single-channel currents were acquired at 10 kHz and filtered at 2 kHz using an Axopatch 200B, a Digidata 1200 interface, and pClamp 5 software.

Generation of stably transfected human embryonic kidney-293 cells. Human embryonic kidney-293 (HEK-293) cells (1×10^6 cells in a 100 mm culture dish) were transfected with 10 μg of cDNA of either α1I, rat α1G (Perez-Reyes et al., 1998), human α1H (Cribbs et al., 1998), or human α1E (Schneider et al., 1994) plus human β_3 (Murakami et al., 1996; a gift from V. Flockerzi). Forty-eight hours after transfection, the cells were suspended in DMEM medium supplemented with G418 (1 gm/l, Life Technologies), 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Individual colonies were isolated with cloning rings. Results were obtained using the following cell lines: α1G, Nr2+; α1H, number 13; and α1Eβ_3 number 1C5. The results from three distinct α1I-transfected cell lines (numbers 11, 19, and 25) were identical and have been pooled.

Electrophysiological analysis of HEK-293 transfected cells. HEK-293 cells were dissociated by digestion with 0.25% trypsin plus 1 mM EDTA (Life Technologies) for 2 min, then diluted 20-fold with DMEM. The cells were triturated, diluted twofold with DMEM, then plated on coverslips. The cells were incubated at least 4 hr and up to 2 d before electrophysiological studies. The internal pipette solution contained the following (in mM): 55 CsCl, 75 $CsSO_4$, 10 $MgCl_2$, 0.1 EGTA, and 10 HEPES, pH adjusted to 7.2 with CsOH. The recording solution contained the following (in mM): 10 $BaCl_2$ solution (or 2 $CaCl_2$), 140 tetraethylammonium (TEA) chloride, 6 CsCl, and 10 HEPES, pH adjusted to 7.4 with TEA-OH. Whole-cell currents were recorded from ruptured patches using an Axopatch 200A amplifier, Digidata 1200 analog-to-digital converter, and pClamp 6.0 software (Axon Instruments). Data were filtered at 1 kHz and digitized at 2 kHz, except for measurement of tail currents that were digitized at 50 kHz. Pipettes were made of TW-150–6 capillary tubing, using a model P-97 Flaming–Brown pipette puller (Sutter Instruments, Novato, CA). Under these solution conditions, the pipette resistance was typically 1.5–2.0 MΩ. Series resistance (correction and prediction) and cell capacitance were compensated ~80%. The average cell capacitance was ~25 pF. The data were not corrected for any residual leak currents. All experiments were performed at room temperature.

Data analysis. Peak currents, integrals, and exponential fits to the electrophysiological data were determined using Clampfit software (Axon Instruments). Conductance was calculated using the Goldman–Hodgkin–Katz equation (Hille, 1992) and the solver function of Excel (Microsoft). Capacitative transients were removed from single-channel records by subtracting null sweeps taken from the same experiment and were then analyzed using Transit (VanDongen, 1996). Single-channel amplitudes were measured by averaging the values obtained from both Gaussian fits to all-points histograms of traces with openings and amplitude histograms of all idealized openings. Fits and graphing of the data were with Prism (GraphPad, San Diego, CA).

RESULTS

Cloning of T-type Ca^{2+} channels began with the identification of an EST clone [IMAGE Consortium (Lennon et al., 1996) clone ID number 44039] as being derived from a novel channel (Perez-Reyes et al., 1998). Homology analysis of the full sequence of this clone (GenBank accession number AF02922) identified a *Caenorhabditis elegans* homolog (GenBank accession number 1017809). The amino acid sequence corresponding to the sixth membrane-spanning region of repeat IV (IVS6) was used to search the EST database using the BLAST algorithm (Altschul et al., 1990), leading to the identification of IMAGE Consortium clones number 50902 (GenBank accession number H19230) and number 402278 (GenBank accession number W76774). Subsequent cloning of the full-length cDNAs for α1G and α1H and mapping of their chromosomal location allowed us to identify these clones as being derived from two genes, *CACNA1G* and *CACNA1H* (Cribbs et al., 1998; Perez-Reyes et al., 1998).

A rat brain cDNA library was screened at low stringency with H19230 (α1G), leading to the isolation of fifty positive plaques. Many of these plaques were not detected in the secondary screening. To test if these lost plaques were derived from α1H, they were rescreened with W76774. Two recombinants were detected with this probe and plaque-purified (clones ME4 and ME5). Sequencing of their cDNA inserts demonstrated that they were similar to each other but clearly different from either α1G or α1H, hence, we called it α1I or Ca_vT.3. This conclusion was supported by having a representative member of each gene cloned from the

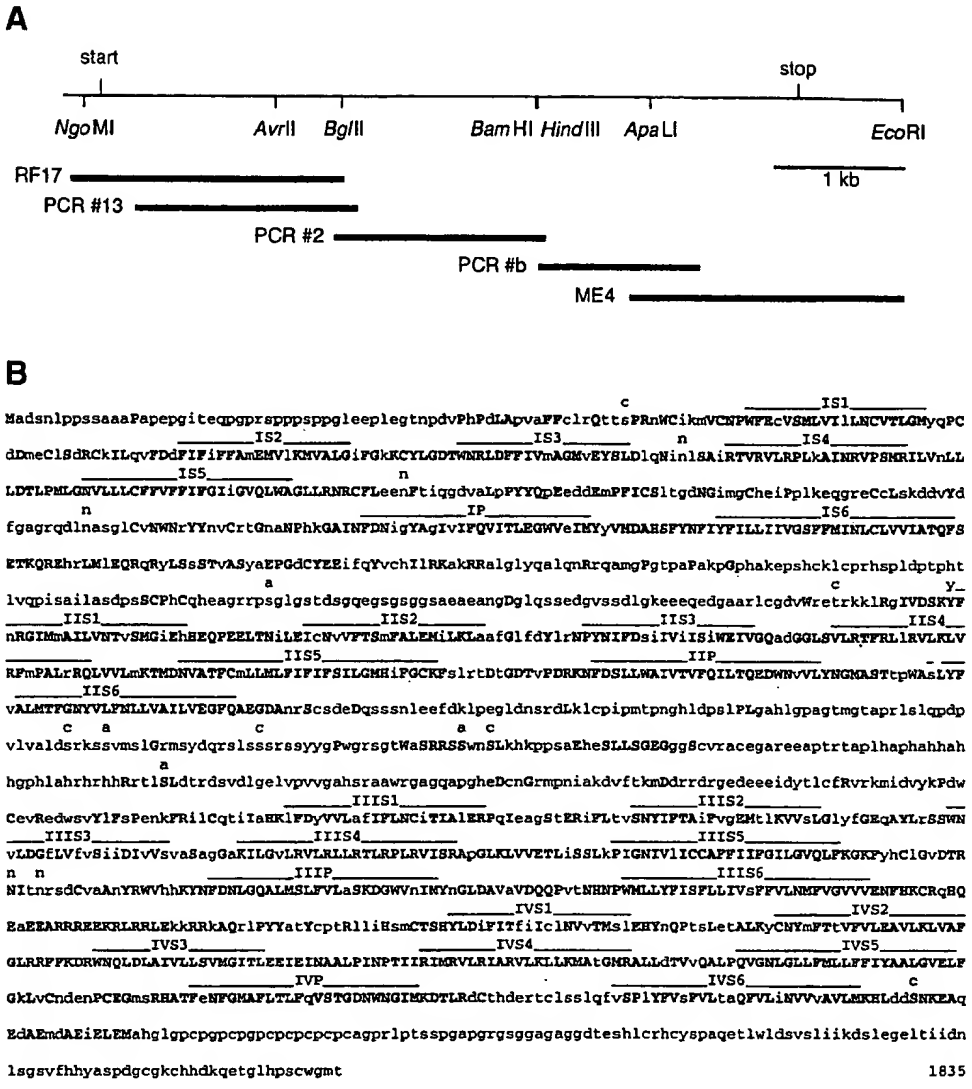

Figure 1. Primary structure and predicted topology of the rat α1I ($Ca_vT.3$). *A*, Schematic showing the location of the restriction enzyme sites and clones used for constructing the full-length cDNA. The cDNA construct was assembled from the following clones: λgt10 clone RF17, *Ngo*M1 (−124)/*Avr*II (1354); PCR clone number 13, *Avr*II (1354)/*Bgl*II (1893); PCR clone number 2, *Bgl*II (1893)/*Bam*HI (3357); a synthetic pair of oligonucleotides, *Bam*HI (3357)/*Hin*dIII(3386); PCR clone b, *Hin*dIII(3386)/*Apa*LI (4327); and λgt10 clone ME4, *Apa*LI (4327)/*Eco*RI (polylinker). *B*, Deduced amino acid sequence of the rat α1I T-type calcium channel. Residues conserved among the rat α1I, rat α1G, and the human α1H are shown in *capitalized bold letters*. Putative membrane-spanning regions are marked *above* the sequence. Analysis of the α1I protein sequence with a modified Prosite database identified the following: four cAMP-dependent protein kinase phosphorylation motifs (R/K-R/K-x-S/T or R/K-R/K-x-x-S/T) all located in the intracellular loops (marked *above* site with the letter *a*); 18 protein kinase C motifs (S/T-x-R/K), eight of which are located intracellularly (marked with *c*); one tyrosine phosphorylation motif (R/K-x-x-x-D-x-x-Y) located at the start of IIS1 (marked with *y*), and seven N-linked glycosylation motifs (N-x-S/T), five of which are in extracellular loops (marked with *n*). *C*, Schematic of the α1I channel showing relationship of loops to the plasma membrane. Each amino acid residue is represented by a *circle*. For diagrammatic purposes, the membrane-spanning regions are modeled as α helices.

rat brain library (UN7, α1G; ME3, α1H; and ME4, α1I). A subsequent search of the HTGS division of the GenBank with the full-length α1G sequence (AF027984) allowed us to recognize the human genomic sequence of α1I (and refer to the gene as *CACNA1I*) on cosmid 20CC7 derived from chromosome 22. This sequence was used to design three sets of PCR primers to clone the cDNA encoding repeats I-III.

The PCR product containing repeat I was used to isolate the 5′ end from the rat brain λgt10 cDNA library. Four clones were isolated, but only RF17 extended into the presumptive 5′ untranslated region. Although clone RF17 contains 278 base pairs at the 5′ end that are enriched with the nucleotides G and C (80% compared with 58% for the coding region), it does not contain an in-frame stop codon. Additional support for our assignment of the start codon comes from the sequence of human cosmid clone 1104E15 (direct submission by J. Sulton, Wellcome Trust Genome Campus). This clone contains a presumptive exon encoding 79 amino acids that are 83% identical to the rat sequence, spanning from the amino terminus sequence to IS1. An in-frame stop codon occurs 171 bp before the start codon we predicted from the rat sequence.

The full-length rat α1I cDNA is composed of 6503 bp (GenBank accession number AF086827). The open reading frame covers 5505 bp, encoding a protein with a predicted molecular weight of 205,198 (Fig. 1*B*). The α1I protein is 59.3% identical to human α1H and 56.9% identical to rat α1G. In contrast, it is only 13–19% identical to the HVA α1 subunits. Most of the residues conserved in all three T channel proteins (Fig. 1*B*) and in HVA α1 subunits are found in the putative membrane-spanning regions. These membrane-spanning regions also share considerable structural homology to voltage-gated K^+ and Na^+ channels (Jan and Jan, 1990), suggesting that the overall topology (Fig. 1*C*) of these channels is similar (Durell et al., 1998). The intracellular loops connecting each repeat and the C terminus are poorly conserved. The T channel α1 proteins contain stretches of histidine and arginine residues, as noted previously for high voltage-activated α1 subunits (Perez-Reyes and Schneider, 1994). In α1G and α1H, this motif occurs in the I-II linker, whereas in α1I it occurs in the II-III linker. In contrast to HVA α1 subunits, the three LVA channels contain a large (107 residues) extracellular loop located between IS5 and the P loop. All other extracellular loops are predicted to be smaller (<35). Although the amino acid sequence is not highly conserved (45%), there are six conserved cysteine residues. Because disulfide bonds are formed in extracellular domains of proteins, this loop may play a role in localizing channels to the cell surface. The C terminus is composed of only 143 amino acids, which is similar in length to human α1H (185), but shorter than either rat α1G (430) or HVA channels such as human α1C (773) or α1E (590). It also contains nine sequential copies of the repeat, TGCCCC, leading to runs of prolines and cysteines. This repetitive element is not found in the human genomic sequence. Analysis of the α1I protein sequence with a modified Prosite database identified the following: four cAMP-dependent protein kinase phosphorylation motifs located in the intracellular loops (Fig. 1*B*), eight protein kinase C motifs located intracellularly, one tyrosine phosphorylation motif located at the start of IIS1, and five N-linked glycosylation motifs on extracellular loops. Motifs for binding β subunits of either G-proteins (Q-x-x-E-R; Chen et al., 1995) or HVA calcium channels (Q-Q-x-E-x-x-L-x-G-Y-x-x-W-I-x-x-x-E; DeWaard et al., 1996) were not identified.

The distribution of α1I mRNA in various rat tissues was determined by Northern blot analysis (Fig. 2). The predominant species detected had a mobility corresponding to 10.5 kb and was only found in brain. Similar results were obtained with two other blots. Minor bands were also observed at 2 and 8 kb. The intensity of the 2 kb band varied between experiments and showed a wider tissue distribution.

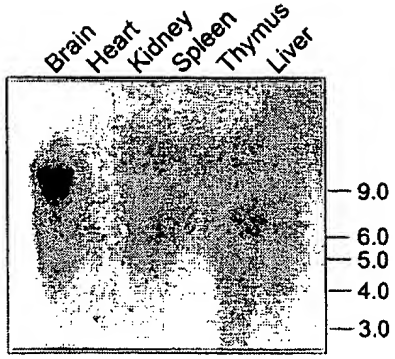

Figure 2. Distribution of α1I mRNA by Northern blot analysis. A rat multiple-tissue blot was probed with ^{32}P-labeled α1I (nucleotides 5142–6197) and exposed for 5 d. Size markers are indicated on the *right* in kilobases. The faint 8 kb bands in kidney and liver were only observed in one experiment and may be caused by contamination of the probe with sequence encoding repeat IV leading to cross-hybridization with α1H (Cribbs et al., 1998). Alternatively, these bands may represent cross-hybridization with a distinct mRNA.

Functional expression of α1I currents was first studied in *Xenopus* oocytes injected with cRNA. Quite surprisingly, the currents activated very slowly, particularly at threshold voltages where the time-to-peak was >150 msec (Fig. 3*A*,*B*). Robust expression (>1 μA) was obtained in most batches of oocytes. Oocytes expressing >2 μA were excluded from the analysis, which reduced the average peak current to −718 ± 146 nA. Kinetics were not affected by BAPTA injection into the oocytes before recording (n = 5), so the data were pooled. This result indicated that there was minimal activation of the Ca^{2+}-activated Cl current. To investigate the possibility of a mutation, the full-length cDNA construct was sequenced. No striking differences were observed in the sequence of α1I as compared with either the human genomic sequences containing *CACNA1I*, (GenBank accession numbers AL022319, AL008716), rat α1G (GenBank accession number AF027984), or human α1H (GenBank accession number AF051946).

When α1I was expressed by stable transfection into HEK-293 cells, the currents were twofold faster (80 msec time-to-peak at threshold; Fig. 3*A*,*C*) than observed in oocytes, but their kinetics were much slower than observed previously for either α1G (Perez-Reyes et al., 1998) or α1H (Cribbs et al., 1998). To compare the gating properties of cloned voltage-gated Ca^{2+} channels, we prepared stably transfected HEK-293 cells of α1I, α1G, α1H, and α1E plus β_3. Robust expression (>1 nA; Fig. 4*A*) was obtained with all cloned channels. Representative current traces obtained during pulses of varying test potentials are shown in Figure 3*C–F*. The peak currents were averaged and plotted versus test potential (Fig. 4*A*). To illustrate the position of these current–voltage curves, the data from each cell were normalized to the largest peak current observed, then averaged (Fig. 4*B*). These results indicated that α1I, α1G, and α1H channels were all activated at low voltages. In contrast, α1Eβ_3 channels required stronger depolarizations (30 mV) to open. To quantitate these differences, conductance was calculated using the Goldman–

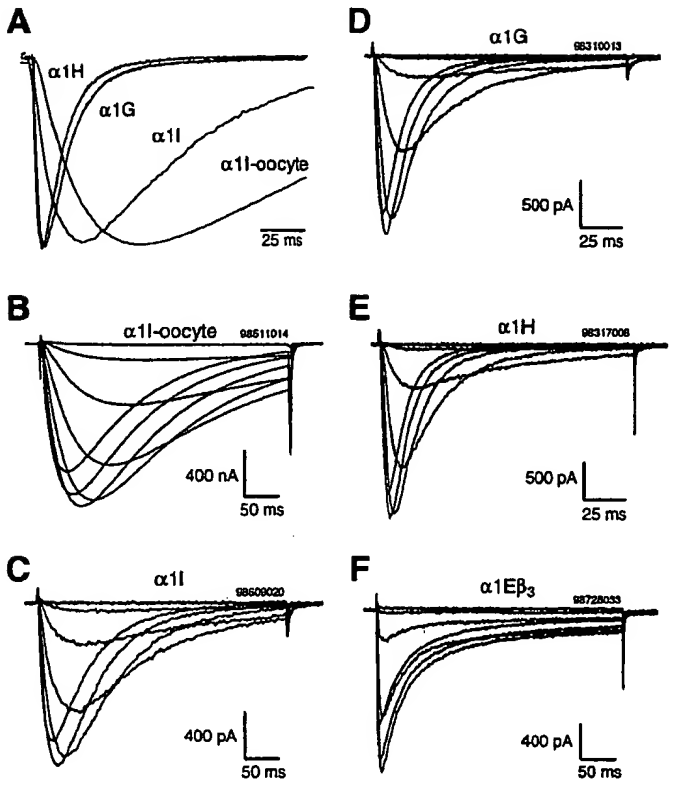

Figure 3. Comparison of the α1I currents to cloned α1G, α1H, and $\alpha 1E\beta_3$ channel currents. Currents were evoked by step depolarizations to varying test potentials from a holding potential of −90 mV. Currents were measured in stably transfected HEK-293 cells using the ruptured patch-clamp method with 10 mM Ba^{2+} as the charge carrier. Also shown are results from *Xenopus* oocytes expressing α1I. *A*, α1I currents expressed in HEK-293 cells and *Xenopus* oocytes were compared with α1G and α1H currents expressed in HEK-293 cells. Currents from the peak of the current–voltage relationship have been scaled and superimposed. Data were taken from the same cells shown in panels *B–F*. *B*, Representative current traces recorded from oocytes injected with α1I-cRNA. Currents were evoked during test pulses that incremented 7 mV with each episode. *C–F*, Representative currents from HEK-293 cells stably transfected with either α1I (*C*), α1G (*D*), α1H (*E*), or $\alpha 1E\beta_3$ (*F*). Currents were elicited by depolarizing 10 mV steps from −90 mV.

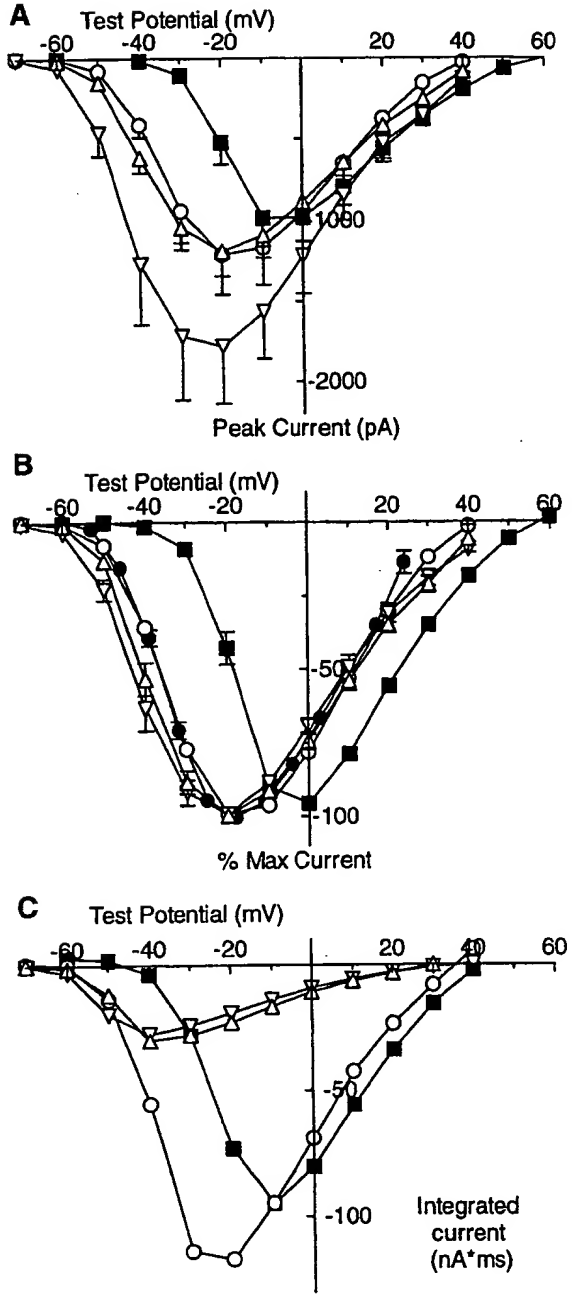

Figure 4. Comparison of the current–voltage (*I–V*) relationships of α1I to those of α1G, α1H, and α1Eβ3. Symbols representing each cloned channel are the same in Figures 4–6: α1G (△), α1H (▽), α1I (○), and $\alpha 1E\beta_3$ (■). *A*, Average peak currents elicited during test pulses to the indicated potentials. Data represent the mean ± SEM from the following number of cells: α1G ($n = 8$), α1H ($n = 6$), α1I ($n = 10$), and $\alpha 1E\beta_3$ ($n = 10$). *B*, The data in *A* were normalized to the peak current observed for each cell then averaged. Also shown is the average data obtained with oocytes injected with α1I (●; $n = 12$). *C*, Integral of the current measured during each test pulse is plotted as a function of test potential. Representative cells were chosen that each expressed 1 nA current at the peak of the *I–V*.

Hodgkin–Katz equation and fit with the Boltzmann equation (see Fig. 6*C*). The values of half-maximal activation ($V_{0.5}$) and slope (k) are presented in Table 1. These results show that each cloned T-type channel activated at slightly different potentials, with α1H being the most negative followed closely by α1G, whereas α1I activated at 7 mV higher test potentials. In contrast, $\alpha 1E\beta_3$ currents activated 15 mV more positive than α1I. These results were obtained using 10 mM Ba^{2+} as the charge carrier. Because of the effects on surface charge screening by such high concentrations of divalent cation (Wilson et al., 1983), we also measured currents under more physiological conditions (2 mM Ca^{2+}; Table 1). The currents through α1I, α1G, and α1H channels also had an apparent reversal potential that was ~15 mV more negative than $\alpha 1E\beta_3$ (Fig. 4*B*). Integrated currents from representative cells, each of which had ~1 nA of peak current, were also plotted as a function of the test potential (Fig. 4*C*). These results showed that α1I caused the biggest influx of Ba^{2+} among the cloned channels.

To measure both activation and inactivation time courses, the pulse was lengthened to 350 msec, and the resulting data were fit with two exponentials (Fig. 5*A,B*). As observed for both native (Huguenard, 1996) and cloned T-type channels (Cribbs et al., 1998; Perez-Reyes et al., 1998), activation and inactivation kinetics were slow near threshold voltages and accelerated with increasing depolarizations, producing a classical criss-crossing pat-

Table 1. Summary of the voltage-dependent properties of cloned calcium channels

	Activation			Inactivation		
	V_{50}	k	τ_{act}	V_{50}	k	τ_{in}
α1I	−24.7 ± 0.2	8.1 ± 0.2	4.7 ± 0.3	−68.3 ± 0.8	−6.3 ± 0.7	55 ± 3
α1G	−28.6 ± 0.9	8.9 ± 0.8	1.8 ± 0.1	−72.4 ± 0.5	−4.8 ± 0.5	15 ± 1
α1H	−31.4 ± 0.9	8.8 ± 0.8	1.7 ± 0.2	−80.9 ± 0.4	−5.5 ± 0.4	14 ± 1
α1Eβ3	−10.9 ± 0.5	6.3 ± 0.4	1.3 ± 0.1	−75.5 ± 0.5	−7.3 ± 0.5	48 ± 2
α1I-2 Ca	−45.0 ± 0.9	7.6 ± 0.8	3.9 ± 0.3	−77.1 ± 0.6	−6.0 ± 0.5	76 ± 10

Average data were fit with the Boltzmann equation to determine the midpoint of voltage dependence (V_{50}, mV) and slope (k). Time constants (in milliseconds) of activation and inactivation were calculated from double exponential fits to the current traces obtained during test pulses to +10 mV.

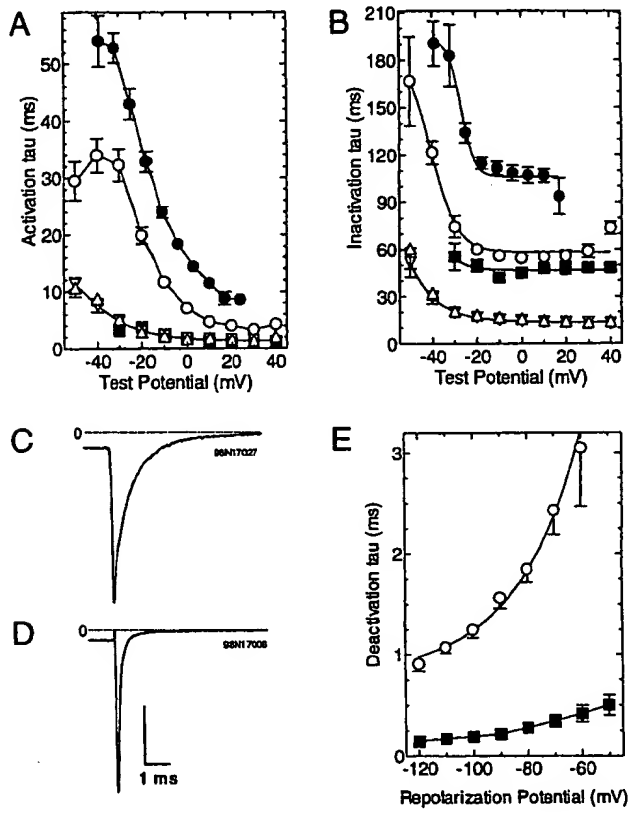

Figure 5. Comparison of the kinetic properties of α1I with those of α1G, α1H, α1I, and α1Eβ$_3$. *A*, *B*, Currents elicited during the *I–V* protocol were fit with two exponentials. Average activation (*A*) and inactivation (*B*) tau values are plotted as a function of test potential. All currents were recorded from HEK-293 cells, except for the data represented by ●, which are from oocytes injected with α1I. Data represent the mean ± SEM from the following number of cells: α1G (△, n = 8), α1H (▽, n = 6), α1I (○, n = 10), α1I in oocytes (●, n = 15), and α1Eβ$_3$ (■, n = 14). *C*, *D*, Representative tail currents from cells expressing either α1I (*C*) or α1Eβ$_3$ (*D*). Currents were evoked by test pulses to either −20 (α1I) or 0 (α1Eβ$_3$) mV, followed by repolarization to −100 mV. *Vertical scale bar* represents 1 (*C*) or 5 nA (*D*). *E*, Data obtained in *C* and *D* were fit with a single exponential. Average deactivation time constants of α1I (n = 4) and α1Eβ$_3$ (n = 4) tail currents were plotted as a function of repolarization potential.

tern (Randall and Tsien, 1997). This pattern was clearly distinct from HVA channels such as α1Eβ$_3$ (Fig. 3*F*) whose activation and inactivation time constants were relatively voltage-independent (Fig. 5*A*,*B*). The current kinetics of α1G and α1H were voltage-dependent and were nearly identical to each other. Similar results were obtained previously with α1G expressed in oocytes (Perez-Reyes et al., 1998) and α1H in transiently transfected HEK-293 cells (Cribbs et al., 1998). In contrast, α1I kinetics were threefold slower in HEK-293 cells and sixfold slower in oocytes.

A second defining feature of T-type Ca^{2+} currents is that they deactivate relatively slowly, producing slowly decaying tail currents after a depolarizing pulse. Representative tail currents for α1I and α1Eβ$_3$ are shown in Figure 5, *C* and *D*, respectively. The data were fit with a single exponential to determine the time constant for deactivation (Fig. 5*E*). These results indicated that α1I channels closed at least sixfold slower than α1Eβ3 channels (at −100 mV, α1I τ, 1.25 ± 0.08 msec; α1Eβ3, 0.19 ± 0.03 msec; n = 4 for both). Fast deactivating tail currents have also been reported previously for both α1Eα$_2$β$_{1a}$ and R-type currents (Williams et al., 1994; Randall and Tsien, 1997).

Inactivation was also studied by applying 5-sec-long prepulses that were terminated by a brief (5 msec) repolarization to close any open channels, then followed by a test pulse to −30 mV to measure channel availability. Representative current traces recorded during prepulses to −50 and −55 mV are shown in Figure 6*A*. Average data were fit with the Boltzmann equation (Fig. 6*B*, Table 1). These results showed that each cloned T-type channel inactivated at slightly different potentials, with α1H inactivating at the lowest potentials, followed by α1G, whereas α1I required potentials that were 15 mV higher. Comparison of α1I channels in HEK-293 cells to those expressed in oocytes indicated that the voltage dependence of activation was nearly identical (Fig. 4*B*) but that inactivation occurred at 7 mV higher potentials in oocytes (V_{50} = −57.7 ± 0.8; n = 7). In contrast, the voltage dependence of α1G was nearly identical in HEK-293 cells, as reported previously for oocytes (Perez-Reyes et al., 1998). The traces in Figure 6*A* were chosen to illustrate that there are voltages at which channels were activated during the prepulse, but they were not completely inactivated, as evidenced by currents evoked during the test pulse. This activity is referred to as a window current and is typically illustrated by the overlap in the steady-state inactivation and activation curves. The resulting window regions for α1I, α1G, α1H, and α1Eβ$_3$ are shown in panels *D–I* of Figure 6. Of the three T-type channels, α1I had the largest window region. In contrast, α1Eβ$_3$ currents did not display a significant window region because inactivation occurred at very negative potentials. The window region is also shown for α1I currents measured with 2 mM Ca^{2+} (Fig. 6*I*). At the peak of the window (−64 mV), ~0.6% of the α1I channels may open (percentage of channels available to gate times the number of channels that gate at that potential).

T-type channels are also defined by their tiny single-channel conductance in saturating concentrations of Ba^{2+} (Fox et al., 1987; Huguenard, 1996). To measure these small currents, we

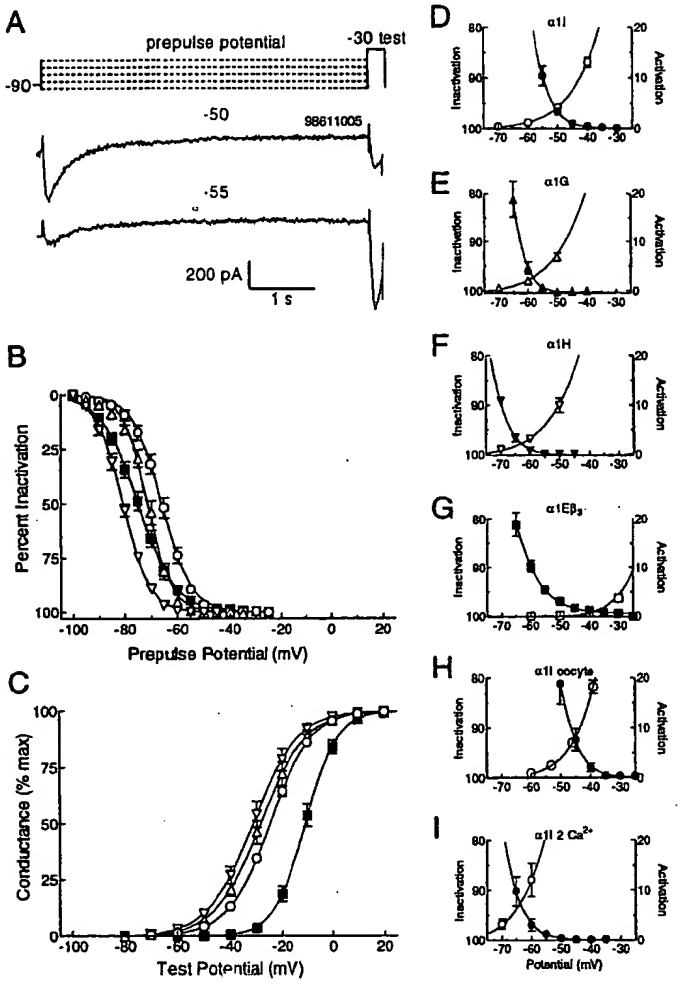

Figure 6. Comparison of steady-state inactivation, activation, and window currents of α1I to those of α1G, α1H, and $\alpha 1E\beta_3$. *A*, The voltage protocol used to measure inactivation is shown *above* representative traces obtained during prepulses to −50 and −55 mV. The protocol also included a short 5 msec repolarization to −90 mV at the end of the prepulse. The time between episodes was 15 sec. *B*, Average percent inactivation was plotted as a function of prepulse voltage. The average data were fit with the Boltzmann equation (*smooth curves*). Data represent the mean ± SEM from the following number of observations: α1G ($\triangle$, $n = 6$), α1H (∇, $n = 8$), α1I (○, $n = 7$), and $\alpha 1E\beta_3$ (■, $n = 12$). *C*, Conductance was calculated using the Goldman–Hodgkin–Katz equation. The data were averaged, then fit with the Boltzmann equation (*smooth curves*). Data represent the mean ± SEM from the following number of observations: α1G ($n = 8$), α1H ($n = 6$), α1I ($n = 8$), and $\alpha 1E\beta_3$ ($n = 14$). *D–I*, Activation and inactivation curves shown in *B* and *C* were overlapped and expanded to show window currents. Data for α1I (*D*), α1G (*E*), α1H (*F*), $\alpha 1E\beta_3$ (*G*), and α1I expressed in oocytes (*H*) were recorded in 10 mM Ba^{2+} solutions. Also shown are data obtained using 2 mM Ca^{2+} as the charge carrier from HEK-293 cells stably transfected with α1I (*I*). *Smooth curves* represent Boltzmann fits to the all the activation data points and to inactivation data points that were >50%.

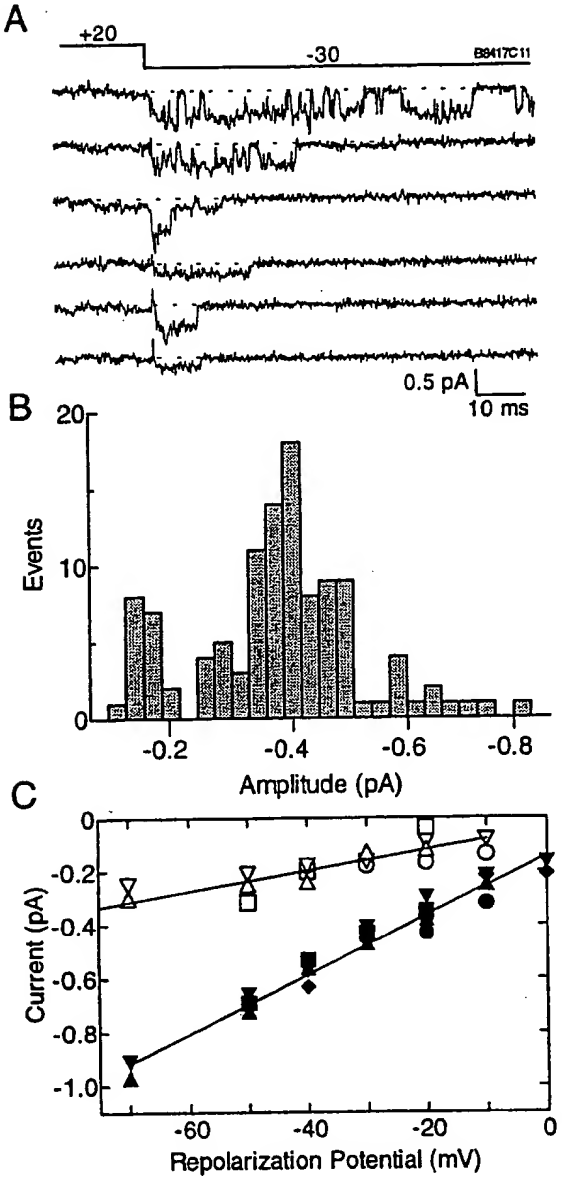

Figure 7. Single-channel currents of α1I measured from *Xenopus* oocytes using the cell-attached patch-clamp method. *A*, Representative traces from a single patch displaying full and subconductance openings of α1I. The voltage protocol contained a prepulse to +20 mV followed by a test pulse to −30 mV. *B*, Channel openings and closings were idealized using Transit to determine the amplitude of channel openings. Data are taken from the same patch shown in *A*. *C*, Single-channel conductance of α1I currents. The amplitudes of single channels were obtained from Gaussian fits to amplitude histograms of idealized openings. The amplitudes were plotted against test potential. Slope conductances were calculated by linear regression through all the data points. Data were obtained from five patches on four oocytes. *Filled symbols* represent full conductance states, whereas *open symbols* represent subconductance states measured from the same patch.

used a tail current protocol that increases the probability of channel opening at negative potentials where the driving force is larger, and hence the currents are larger. Representative sweeps were chosen to illustrate that channel openings occur in bursts and to show the presence of a subconductance state (Fig. 7*A*). The third trace in Figure 7*A* shows a channel closing from the full to a subconductance state, whereas traces 4 and 6 show openings to the subconductance state. The data were idealized with the Transit algorithm (VanDongen, 1996), and the amplitude of the idealized openings were plotted in Figure 7*B*. This plot showed that channels opened to two distinct amplitudes. Gaussian fits to these histograms were used to determine the amplitude of the openings, then plotted as a function of repolarization potential. The data were then fit by linear regression to determine the slope conductance. The conductance of the small openings was 3.9 ± 0.5 pS, whereas the larger openings had a conductance of 11.0 ±

0.5 pS. The current at 0 mV for the full conductance state was −0.15 pA.

DISCUSSION

The present study describes the cloning and expression of a novel member of the T-type Ca^{2+} channel family. Discovery of this family of genes was made possible by the development of normalized cDNA libraries (Soares et al., 1994), systematic sequencing of clones from these libraries, and free access to their DNA sequences (Lennon et al., 1996). We cloned the first cDNA fragment of α1I by low-stringency screening of a rat brain cDNA library with two IMAGE Consortium clones that encoded either α1G or α1H. The cloning project was greatly facilitated by efforts to sequence the human genome, in particular the work performed by the Wellcome Trust Genome Campus, which led to partial sequencing of the human α1I gene, *CACNA1I*, and its localization on human chromosome 22q12.3–13.2.

Sequence homology can be used to subdivide the family of voltage-gated Ca^{2+} channel α1 subunits into three subfamilies: (1) T-type (G, H, I); (2) L-type (S, C, D, F); and (3) non-L-type HVA (A, B, E). Electrophysiological characterization of these cloned channels has revealed considerable functional differences between the members of each subfamily. For example, α1S encodes a slow Ca^{2+} channel (Perez-Reyes et al., 1989), whose main physiological role is as a voltage sensor, coupling depolarization to skeletal muscle contraction (Stern et al., 1997). Similarly α1E has unique characteristics of inactivation gating and permeation that set it apart from α1A and α1B (Soong et al., 1993; Bourinet et al., 1996). In the T-type subfamily, it is α1I that stands apart. It encodes a slowly activating and inactivating Ca^{2+} channel that gates in voltage ranges similar to, but higher than the other two cloned T channels. It can be classified as a T-type channel by virtue of its slowly deactivating tail currents and tiny single-channel conductance in 115 mM $BaCl_2$.

The deduced amino acid sequence of α1I is ~58% identical to either α1G or α1H, but only ~15% identical to the HVA α1 subunits. The regions of least conservation between the T-type channels are their intracellular loops. The III-IV linker is an exception because it is 75% identical among the three T channel proteins. Perhaps this high degree of sequence identity is caused by conservation of function as observed in voltage-gated Na^+ channels where the III-IV linker plays a role in fast inactivation (Catterall, 1995). A role of the intracellular linkers in inactivation was postulated before the structure of T channels was even deduced (Miller and Hu, 1995).

Three major conclusions from our expression studies with α1I are: one, it encodes a T-type Ca^{2+} channel with a unique voltage dependence; two, it encodes a slow channel; and three, its activity is dependent on the expression system. Expression in both heterologous expression systems demonstrated that α1I encoded low voltage-activated currents. The threshold voltage for channel activation was −60 mV (in 10 mM Ba^{2+}), which was similar to what we have observed for α1G and α1H (Cribbs et al., 1998; Perez-Reyes et al., 1998). Expression in *Xenopus* oocytes led to α1I currents that were as slow as those observed for the L-type channels of skeletal muscle (Garcia et al., 1992). In contrast, α1I currents from transfected HEK-293 cells activated and inactivated much more quickly. In addition, the voltage dependence of steady-state inactivation differed between these two expression systems, with the oocyte currents requiring 8 mV higher depolarizations. The reason for this discrepancy is under investigation. A plausible explanation is that HEK-293 cells, or oocytes, express a subunit of T-type channels that can influence kinetics and steady-state inactivation. High voltage-activated Ca^{2+} channels are multisubunit complexes, which in addition to α1 subunits, also contain at least two and sometimes three auxiliary subunits, $\alpha_2\delta$, β, and γ. All of these subunits have been reported to modulate channel properties (Perez-Reyes and Schneider, 1994); by analogy, it is likely that LVA channels also have accessory subunits. It should be noted that HEK-293 cells were originally derived from human kidney (Graham et al., 1977), which is the tissue with the highest expression of α1H (Cribbs et al., 1998). It is also interesting to speculate that the newly identified γ2 subunit may be a T-type channel subunit (Letts et al., 1998). Mutations in the γ2 gene are thought to be responsible for the absence epilepsy phenotype of the *stargazer* mouse. Similarly, it has been suggested that increased T channel activity may cause absence epilepsy in rats (Tsakiridou et al., 1995).

Three criteria can be used to define T-type channels, their opening at membrane potentials near the resting membrane potential of most cells (LVA), their slow closing after a depolarization (SD), and their tiny (T) single-channel conductance in saturating concentrations of Ba^{2+} (Matteson and Armstrong, 1986; Carbone and Lux, 1987; Fox et al., 1987). T-type channels also have a distinctive criss-crossing set of current traces obtained during the *I–V* protocol (Randall and Tsien, 1997). Despite its slow kinetics, α1I still produces this distinctive pattern. This pattern is the result of the voltage-dependence of T channel kinetics, where activation kinetics are determined by the latency to first opening and by an inactivation process that is tightly coupled to activation (Carbone and Lux, 1987; Droogmans and Nilius, 1989; Chen and Hess, 1990; Miller and Hu, 1995). Despite the slower activation kinetics than either α1G or α1H, α1I deactivates with a similar time course, producing a slow tail current. HVA channels, such as α1E, close at least sixfold faster. The exact mechanism by which T channels, which have a mean open time of ~1 msec, produce such a slow tail has not been fully characterized.

One of the early methods for separating LVA from HVA currents was to record currents from holding potentials of −90 and −40 mV, then subtract the currents. This was useful because in many cells only LVA currents inactivate at −40 mV. Recent studies suggest that some HVA channels inactivate at lower potentials than LVA channels, notably the R-type (Randall and Tsien, 1997) and the cloned α1E (Fig. 6). Therefore steady-state inactivation is no longer a defining feature of LVA channels. Among the cloned T channels, we find a 15 mV difference between the subtypes, with α1I requiring the highest prepulse potentials. Because activation of α1I is also shifted to more depolarized potentials, this leads to its having the largest window currents among the three cloned T channels. Notably, this window current occurs very close to the resting membrane potential of most cells, suggesting that α1I may play a role in determining resting concentrations of intracellular Ca^{2+}. Window currents are an essential property of channels involved in pacemaker activity and play a critical role in the integration of synaptic potentials (Williams et al., 1997).

The single-channel conductance of native T channels ranges between 5 and 9 pS (Huguenard, 1996). Similarly, we found that α1G had a single-channel conductance of 7.5 pS and that α1H was slightly smaller, 5.3 pS (Cribbs et al., 1998; Perez-Reyes et al., 1998). The conductance of α1I was significantly larger (11 pS), approaching the value determined for rat α1E, 12.5 pS (Bourinet et al., 1996). In addition, rat α1E conducts Ba^{2+}, Ca^{2+}, and Sr^{2+}

equally, as observed for native T channels (Shuba et al., 1991). Although α1E and α1I have similar slope conductances, the single-channel amplitudes are very different at 0 mV (α1E, −0.5 pA; α1I, −0.15 pA) because they have distinct reversal potentials. Evidence for the different reversal potentials was presented at the whole-cell level (Fig. 4*B*). Measurement of the conductance of cloned T channels is complicated by the presence of a subconductance state. Evidence that these smaller openings are caused by the cloned T channels and not an endogenous oocyte channel was the following: (1) endogenous Ca^{2+} channels were not detected at the whole-cell level in these batches of oocytes, (2) endogenous channels generate −0.5 pA current at 0 mV (Lacerda et al., 1994), (3) transitions between the full and subconductance states are clearly visible (Fig. 7), and (4) both types of openings disappear when the holding potential is shifted to −40 mV (results not shown). The presence of subconductance states for native cardiac T channels (Droogmans and Nilius, 1989) and cloned HVA channels (Meir and Dolphin, 1998) have been noted.

The discovery of a clone encoding slow T-type channels may have been predicted from studies in native cells. Slow T-type channels have been described in neurons isolated from various rat thalamic nuclei, such as the reticular (Huguenard and Prince, 1992), laterodorsal (Tarasenko et al., 1997), and lateral habenula (Huguenard et al., 1993). They have also been described in a dorsal root ganglion–neuroblastoma hybrid cell line (Dolphin, 1998). These native T currents inactivated with nearly identical time constants as observed for α1I (55 msec). We suggest that these slow T channels are encoded by α1I. Support for this hypothesis is provided by the expression of α1I mRNA in these same brain regions (Talley et al., 1999). Notably, α1I is abundantly expressed in the thalamic reticular nucleus and lateral habenula. In addition, slow thalamic T channels required stronger depolarizations for channel opening than the fast T currents (Huguenard and Prince, 1992; Tarasenko et al., 1997). Similarly, we find that α1I gates at less negative potentials than either α1G or α1H. One notable difference is that the slow thalamic T current inactivated at more negative potentials than the fast, whereas we find the opposite result. However, we also found that the voltage dependence of inactivation varied between expression systems, suggesting this property may be affected by auxiliary subunits. Injection of thalamic–hypothalamic mRNA into *Xenopus* oocytes has been reported to produce LVA channels (Dzhura et al., 1996). The relationship of these currents to α1I is not clear, because these currents inactivated much more slowly.

Knowledge of the distribution and functional properties of the three T channels should lead to a greater understanding of their physiological roles. T channels are thought to play a pacemaker role in the genesis of rebound burst firing which, through reciprocal connections, can lead to oscillations and resonance of neuronal circuits (Huguenard, 1996). Burst firing of thalamic T channels is thought to be important in the transition to sleep and in the pathophysiology of epilepsy (McCormick and Bal, 1997). The ability of many antiepileptics to inhibit T channel activity led to the hypothesis that they may be involved in epilepsy (Coulter et al., 1990). Support for this hypothesis came from the observation that T channel activity of thalamic reticular neurons was increased 50% in GAERS (Tsakiridou et al., 1995), a well defined rat model of absence epilepsy (Vergnes and Marescaux, 1994). Cloning of T channels and the ability to express these channels at high density in stably transfected cells should provide an assay to study the pharmacological properties of T channels and may lead to the development of a new generation of antiepileptic drugs.

REFERENCES

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410.

Bech-Hansen NT, Naylor MJ, Maybaum TA, Pearce WG, Koop B, Fishman GA, Mets M, Musarella MA, Boycott KM (1998) Loss-of-function mutations in a calcium-channel α1-subunit gene in Xp11.23 cause incomplete X-linked congenital stationary night blindness. Nat Genet 19:264–267.

Bourinet E, Zamponi GW, Stea A, Soong TW, Lewis BA, Jones LP, Yue DT, Snutch TP (1996) The α_{1E} calcium channel exhibits permeation properties similar to low-voltage-activated calcium channels. J Neurosci 16:4983–4993.

Carbone E, Lux HD (1987) Single low-voltage-activated calcium channels in chick and rat sensory neurones. J Physiol (Lond) 386:571–601.

Catterall WA (1995) Structure and function of voltage-gated ion channels. Annu Rev Biochem 64:493–531.

Chen CF, Hess P (1990) Mechanism of gating of T-type calcium channels. J Gen Physiol 96:603–630.

Chen J, DeVivo M, Dingus J, Harry A, Li J, Sui J, Carty DJ, Blank JL, Exton JH, Stoffel RH, Inglese J, Lefkowitz RJ, Logothetis DE, Hildebrandt J, Iyengar R (1995) A region of adenylyl cyclase 2 critical for regulation by G protein βγ subunits. Science 268:1166–1169.

Coulter DA, Huguenard JR, Prince DA (1990) Differential effects if petit mal anticonvulsants and convulsants on thalamic neurones: calcium current reduction. Br J Pharmacol 100:800–806.

Cribbs LL, Lee J-H, Yang J, Satin J, Zhang Y, Daud A, Barclay J, Williamson MP, Fox M, Rees M, Perez-Reyes E (1998) Cloning and characterization of α1H from human heart, a member of the T-type calcium channel gene family. Circ Res 83:103–109.

DeWaard M, Scott VES, Pragnell M, Campbell KP (1996) Identification of critical amino acids involved in α_1-β interaction in voltage-dependent Ca^{2+} channels. FEBS Lett 380:272–276.

Dolphin AC (1998) Properties and modulation of T-type currents in dorsal root ganglia and ND7–23 cells: comparison with α1E currents expressed in COS-7 cells. In: Low voltage-activated T-type calcium channels (Tsien RW, Clozel J-P, Nargeot J, eds), pp 269–278. Chester, UK: Adis International.

Droogmans G, Nilius B (1989) Kinetic properties of the cardiac T-type calcium channel in the guinea-pig. J Physiol (Lond) 419:627–650.

Durell SR, Hao Y, Guy HR (1998) Structural models of the transmembrane region of voltage-gated and other K^+ channels in open, closed, and inactivated conformations. J Struct Biol 121:263–284.

Dzhura IO, Naidenov VG, Lyubanova OP, Kostyuk PG, Shuba YM (1996) Characterization of hypothalamic low-voltage-activated Ca channels based on their functional expression in *Xenopus* oocytes. Neuroscience 70:729–738.

Ellinor PT, Zhang J-F, Randall AD, Zhou M, Schwarz TL, Tsien RW, Horne WA (1993) Functional expression of a rapidly inactivating neuronal calcium channel. Nature 363:455–458.

Fox AP, Nowycky MC, Tsien RW (1987) Single-channel recordings of three types of calcium channels in chick sensory neurones. J Physiol (Lond) 394:173–200.

Garcia J, McKinley K, Appel SH, Stefani E (1992) Ca^{2+} current and charge movement in adult single human skeletal muscle fibres. J Physiol (Lond) 454:183–196.

Graham FL, Smiley J, Russell WC, Nairn R (1977) Characteristics of a human cell line transformed by DNA from adenovirus type 5. J Gen Virol 36:59–72.

Hille B (1992) Ionic channels of excitable membranes. Sunderland, MA: Sinauer.

Huguenard JR (1996) Low threshold calcium currents in central nervous system neurons. Annu Rev Physiol 58:329–348.

Huguenard JR, Prince DA (1992) A novel T-type current underlies prolonged Ca(2+)-dependent burst firing in GABAergic neurons of rat thalamic reticular nucleus. J Neurosci 12:3804–3817.

Huguenard JR, Gutnick MJ, Prince DA (1993) Transient Ca2+ currents in neurons isolated from rat lateral habenula. J Neurophysiol 70:158–166.

Jan LY, Jan YN (1990) A superfamily of ion channels. Nature 345:672.

Lacerda AE, Perez-Reyes E, Wei X, Castellano A, Brown AM (1994) T-type and N-type calcium channels of *Xenopus* oocytes: evidence for specific interactions with beta subunits. Biophys J 66:1833–1843.

Lennon G, Auffray C, Polymeropoulus M, Soares MB (1996) The I.M.A.G.E. consortium: an integrated molecular analysis of genomes and their expression. Genomics 33:151–152.

Leonard JP, Snutch TP (1991) The expression of neurotransmitter receptors and ion channels in *Xenopus* oocytes. In: Molecular neurobiology: a practical approach (Chad J, Wheal H, eds), pp 161–182. Oxford: IRL.

Letts VA, Felix R, Biddlecome GH, Arikkath J, Mahaffey CL, Valenzuela A, Bartlett II FS, Mori Y, Campbell KP, Frankel WN (1998) The mouse stargazer gene encodes a neuronal Ca^{2+}-channel γ subunit. Nat Genet 19:340–347.

Liman ER, Tytgat J, Hess P (1992) Subunit stoichiometry of a mammalian K+ channel determined by construction of multimeric cDNAs. Neuron 9:861–871.

Llinas R, Jahnsen H (1982) Electrophysiology of mammalian thalamic neurons *in vitro*. Nature 297:406–408.

Matteson DR, Armstrong CM (1986) Properties of two types of calcium channels in clonal pituitary cells. J Gen Physiol 87:161–182.

McCormick DA, Bal T (1997) Sleep and arousal: thalamocortical mechanisms. Annu Rev Neurosci 20:185–215.

Meir A, Dolphin AC (1998) Known calcium channel α_1 subunits can form low threshold small conductance channels with similarities to native T-type channels. Neuron 20:341–351.

Methfessel C, Witzemann V, Takahashi T, Mishina M, Numa S, Sakmann B (1986) Patch clamp measurements on *Xenopus laevis* oocytes: currents through endogenous channels and implanted acetylcholine receptor and sodium channels. Pflügers Arch 407:577–588.

Miller A, Hu B (1995) A molecular model of low-voltage-activated calcium conductance. J Neurophysiol 73:2349–2356.

Murakami M, Wissenbach U, Flockerzi V (1996) Gene structure of the murine calcium channel β3 subunit, cDNA and characterization of alternative splicing and transcription products. Eur J Biochem 236:138–143.

Ophoff RA, Terwindt GM, Frants RR, Ferrari M (1998) P/Q-type Ca^{2+} channel defects in migraine, ataxia and epilepsy. Trends Pharmacol Sci 19:121–127.

Perez-Reyes E, Schneider T (1994) Calcium channels: structure, function, and classification. Drug Dev Res 33:295–318.

Perez-Reyes E, Kim HS, Lacerda AE, Horne W, Wei XY, Rampe D, Campbell KP, Brown AM, Birnbaumer L (1989) Induction of calcium currents by the expression of the alpha 1-subunit of the dihydropyridine receptor from skeletal muscle. Nature 340:233–236.

Perez-Reyes E, Cribbs LL, Daud A, Lacerda AE, Barclay J, Williamson MP, Fox M, Rees M, Lee J-H (1998) Molecular characterization of a neuronal low voltage-activated T-type calcium channel. Nature 391:896–900.

Randall AD, Tsien RW (1997) Contrasting biophysical and pharmacological properties of T- type and R-type calcium channels. Neuropharmacology 36:879–893.

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor, MA: Cold Spring Harbor Laboratory.

Schneider T, Wei X, Olcese R, Costantin JL, Neely A, Palade P, Perez-Reyes E, Qin N, Zhou J, Crawford GD, Smith RG, Appel SH, Stefani E, Birnbaumer L (1994) Molecular analysis and functional expression of the human type E α_1 subunit. Receptors Channels 2:255–270.

Shuba YM, Teslenko VI, Savchenko AN, Pogorelaya NH (1991) The effect of permeant ions on single calcium channel activation in mouse neuroblastoma cells: ion-channel interactions. J Physiol (Lond) 443:25–44.

Soares MB, Bonaldo MDF, Jelene P, Su L, Lawton L, Efstratiadis A (1994) Construction and characterization of a normalized cDNA library. Proc Natl Acad Sci USA 91:9228–9232.

Soong TW, Stea A, Hodson CD, Dubel SJ, Vincent SR, Snutch TP (1993) Structure and functional expression of a member of the low voltage-activated calcium channel family. Science 260:1133–1136.

Stern MD, Pizarro G, Rios E (1997) Local control model of excitation-contraction coupling in skeletal muscle. J Gen Physiol 110:414–440.

Strom TM, Nyakatura G, Apfelstedt-Sylla E, Hellebrand H, Lorenz B, Weber BHF, Wutz K, Gutwillinger N, Ruther K, Drescher B, Sauer C, Zrenner E, Meitinger T, Rosenthal A, Meindl A (1998) An L-type calcium-channel gene mutated in incomplete X-linked congenital stationary blindness. Nat Genet 19:260–263.

Talley EM, Cribbs LL, Lee J-H, Daud A, Perez-Reyes E, Bayliss D (1999) Differential distribution of three members of a gene family encoding low voltage-activated (T-type) calcium channels. J Neurosci 19:1895–1911.

Tarasenko AN, Kostyuk PG, Eremin AV, Isaev DS (1997) Two types of low-voltage-activated Ca2+ channels in neurones of rat laterodorsal thalamic nucleus. J Physiol (Lond) 499:77–86.

Tsakiridou E, Bertollini L, de Curtis M, Avanzini G, Pape HC (1995) Selective increase in T-type calcium conductance of reticular thalamic neurons in a rat model of absence epilepsy. J Neurosci 15:3110–3117.

VanDongen AMJ (1996) A new algorithm for idealizing single ion channel data containing multiple unknown conductance levels. Biophys J 70:1303–1315.

Vergnes M, Marescaux C (1994) Pathophysiological mechanisms underlying genetic absence epilepsy in rats. In: Idiopathic generalized epilepsies: clinical, experimental, and genetic aspects (Malafosse A, Genton P, Hirsch E, Marescaux C, Broglin D, Bernasconi R, eds), pp 151–168. London: John Libbey & Co.

Williams ME, Marubio LM, Deal CR, Hans M, Brust PF, Philipson LH, Miller RJ, Johnson EC, Harpold MM, Ellis SB (1994) Structure and functional characterization of neuronal alpha 1E calcium channel subtypes. J Biol Chem 269:22347–22357.

Williams SR, Toth TI, Turner JP, Hughes SW, Crunelli V (1997) The "window" component of the low threshold Ca2+ current produces input signal amplification and bistability in cat and rat thalamocortical neurones. J Physiol (Lond) 505:689–705.

Wilson DL, Morimoto K, Tsuda Y, Brown AM (1983) Interaction between calcium ions and surface charge as it relates to calcium currents. J Membr Biol 72:117–130.

Zamponi GW, Bourinet E, Snutch TP (1996) Nickel block of a family of neuronal calcium channels: subtype- and subunit-dependent action at multiple sites. J Membr Biol 151:77–90.

Characterization of Ethosuximide Reduction of Low-Threshold Calcium Current in Thalamic Neurons

Douglas A. Coulter, PhD, John R. Huguenard, PhD, and David A. Prince, MD

The mechanism by which ethosuximide reduces thalamic low-threshold calcium current (LTCC) was analyzed using voltage-clamp techniques in acutely isolated ventrobasal complex neurons from rats and guinea pigs. The ethosuximide-induced reduction of LTCC was voltage dependent: it was most pronounced at more-hyperpolarized potentials and did not affect the time course of activation or inactivation of the current. Ethosuximide reduced LTCC without altering the voltage dependence of steady-state inactivation or the time course of recovery from inactivation. Dimethadione reduced LTCC by a similar mechanism, while valproic acid had no effect on LTCC. We conclude that ethosuximide reduction of LTCC in thalamic neurons is consistent with a reduction in the number of available LTCC channels or in the single LTCC channel conductance, perhaps indicating a direct channel-blocking action of this drug. Given the importance of LTCC in thalamic oscillatory behavior, a reduction in this current by ethosuximide would be a mechanism of action compatible with the known anticonvulsant effects of this drug in typical absence seizures.

Coulter DA, Huguenard JR, Prince DA. Characterization of ethosuximide reduction of low-threshold calcium current in thalamic neurons. Ann Neurol 1989;25:582–593

Ethosuximide is an anticonvulsant frequently used in the treatment of typical generalized absence (petit mal) epilepsy [1, 2]. In fact, its anticonvulsant action is specific for this type of epilepsy; it is ineffective in the treatment of other types of generalized seizures and against all types of partial seizures [1, 2]. However, few cellular actions of ethosuximide have been described that are consistent with its specific anticonvulsant effects (reviewed in [3]). Characterization of the cellular mechanism of action of ethosuximide could have direct bearing on our understanding of the pathogenesis of petit mal epilepsy, and could also aid in the development of other anticonvulsant drugs.

Evidence from depth recordings in petit mal patients [4], and extracellular recordings in animal models of petit mal [5–7], indicates that the 3-Hz spike-wave rhythms in the electroencephalogram, characteristic of absence seizures, are generated by rhythmic reverberating interactions between mutually interconnected thalamic and cortical areas. In different experimental models of petit mal the thalamus may either lead or follow the cortex in the genesis of these spike-wave rhythms. In rat genetic models of petit mal the discharge in the thalamus may lead that in the cortex [7], whereas in feline generalized penicillin epilepsy the reverse is true [5, 6]. However, what is clear in all of these models, and in recordings from petit mal patients [4], is that the thalamus plays an important role in the generation of 3-Hz spike-wave rhythms characteristic of petit mal epilepsy.

Most studies of the cellular actions of ethosuximide have employed cultured cortical mammalian [8] and spinal neurons [8]; in some cases neurons from invertebrate species have been used [9]. No cellular mechanisms consistent with the anticonvulsant action of ethosuximide have been demonstrated in these types of cells when the drug is applied in concentration ranges appropriate for its clinical action [2, 3]. We chose to examine ethosuximide actions on thalamic neurons because of the important role of the thalamus in generation of petit mal epilepsy. Neurons in virtually all areas of the thalamus are characterized physiologically by the presence of a large-amplitude, low-threshold calcium spike [10–14]. This conductance underlies bursts of action potentials generated in thalamic neurons and plays an important role in thalamic oscillatory behavior (e.g., in the generation of thalamic sleep spindles, as reviewed in [14]).

We have previously shown that the petit mal anticonvulsant ethosuximide reduces the low-threshold calcium current (LTCC) underlying the low-threshold calcium spike in rodent thalamic neurons in vitro [15]. In the present study, we further examined this action of ethosuximide and also tested the effects of another

From the Department of Neurology, Stanford University Medical Center, Stanford, CA.

Received Oct 10, 1988. Accepted Dec 8, 1988.

Address correspondence to Dr Coulter, Department of Neurology, Rm M016, Stanford University Medical Center, Stanford, CA 94305.

specific petit mal anticonvulsant, dimethadione, on calcium currents in thalamic neurons. Our goal was to investigate the mechanism by which these anticonvulsants reduce the LTCC.

Methods

Dissociation

All experiments were performed on thalamic neurons acutely isolated from the ventrobasal complex of rats and guinea pigs (ages 1 day–adult) using the methods described by Kay and Wong [16]. Animals were anaesthetized with pentobarbital and decapitated. A portion of the brain containing the thalamus was removed, trimmed, and glued to the stage of a vibratome (Lancer) for slicing. Thalamic slices (600 μm thick) were then cut and placed in an oxygenated PIPES (piperazine-N, N'-bis[2 ethanesulphonic acid])-buffered solution containing trypsin (0.8 mg/ml, Sigma, St Louis, MO) for 45 minutes to 1 hour. The PIPES solution contained the following (in mM): NaCl, 120; KCl, 5; $CaCl_2$, 1; $MgCl_2$, 1; D-glucose, 25; PIPES 20 (pH 7.0). Slices were then removed from the enzyme solution, rinsed, cut into chunks, and triturated using fire-polished Pasteur pipettes. Cells were plated onto culture dishes and stored in an oxygenated incubator for 0.5 to 12 hours prior to use.

Recording

Patch recordings of calcium currents were made using the whole-cell voltage-clamp method described by Hamill and colleagues [17]. The intracellular solution used to isolate calcium currents was a variation of one previously described [18] and consisted of the following (in mM): Trizma phosphate (dibasic), 110; Trizma base, 28; ethylene glycol bis-(β aminoethylether)-N, N, N', N'-tetraacetic acid, 11; $MgCl_2$, 2; $CsCl_2$, 0.5; Na_2–adenosine triphosphate, 4; pH 7.35. The external solution was composed of the following (in mM): NaCl, 155; KCl, 3; $MgCl_2$, 1; $CaCl_2$, 3; HEPES-Na^+, 10; tetrodotoxin, 0.0005; pH 7.4. All recordings were conducted at room temperature (20–22°C). A 8-mV liquid junction potential was measurable between the electrode and bath solutions, so all voltage command levels were compensated accordingly. Patch electrodes were pulled on a List L/M-3P-A puller (Darmstadt, FRG) using a two-stage pull, and had resistances of 6 to 8 MΩ. Currents were monitored either with a List L/M-EPC-7 or with an Axopatch 1A (Axon Instruments, Burlingame, CA) and filtered at 5 KHz with an 8-pole Bessel filter prior to digitization. All data were stored and analyzed using a DEC PDP-11/73 computer with a Cheshire data interface (Indec, Sunnyvale, CA). A backup copy of most data was recorded on videotape using a pulse-code modulated interface (Neurocorder, Neurodata Instruments, New York, NY). Leak and capacitance currents are subtracted from active currents in all figures.

Series Resistance Errors and Escape

Compensation circuitry was used whenever possible to minimize potential series resistance errors. This circuitry could compensate 90% of the series resistance. Typical series resistance (R_s) errors with a 10 MΩ R_s (compensated to 1 mΩ) and a 500 pA current would be 0.5 mV. When drugs were applied using bath perfusion with high flow rates (for example, in obtaining ethosuximide dose-response data), fluctuations in solution level often induced oscillations due to the R_s compensation circuitry. For this reason, R_s compensation was not used in these instances, and the potential R_s error therefore increased to 5 mV. Voltages during command potentials eliciting calcium currents were judged to be well controlled based on smooth voltage-dependent activation and activation and inactivation kinetics, which were independent of current magnitude (for example, see current traces in steady-state inactivation data).

Drug Concentrations and Method of Application

All anticonvulsants were applied in concentration ranges that were clinically relevant (i.e., in concentrations that are achieved as free serum levels in epileptic patients medicated with a particular anticonvulsant). For ethosuximide and dimethadione this concentration range was 280 to 700 μM (40–100 μg/ml) [1, 2] and 5 to 9 mM (700–1,200 μg/ml) [19, 20], respectively. Drugs were applied either by changing the extracellular bathing medium or by perfusion onto the cell produced by applying pressure to the back of drug-containing broken micropipettes (tip diameter 2–4 μm).

Results

Results presented in this paper are summarized from recordings of drug effects in 143 cells: 80 from rat, and 63 from guinea pig, ventrobasal complex neurons. Results from the two species were indistinguishable and so were pooled for subsequent data analysis.

Thalamic Calcium Currents

It was possible to identify at least two, and possibly three, distinct calcium currents in whole-cell voltage-clamp recordings from thalamic neurons on the basis of activation and inactivation kinetics, voltage thresholds, and pharmacological sensitivity [21]. Figure 1 illustrates the properties of the two largest, most easily distinguishable calcium currents, which were the focus of our studies of anticonvulsant pharmacological action. At holding potentials of −100 mV, depolarizing voltage commands to −60 mV or more positive potentials elicited a transient calcium current that inactivated rapidly during the time course of the 200-msec voltage command (see Fig 1A, left traces). This current was similar to the "T" [22], type I [23], or l.v.a. [24] calcium current described by others, and will henceforth be called the low-threshold calcium current. The LTCC became larger with increasing voltage commands and peaked in isolation during voltage commands to −40 mV (see Fig 1A, left traces and Fig 1B, IV plot). During voltage commands to −30 mV or more positive potentials, a sustained noninactivating or slowly inactivating calcium current began to appear that peaked at 0 mV (see Fig 1B, right traces and IV plot). This current was similar to the "L" [22], type II [23], or h.v.a. [24] calcium current described by

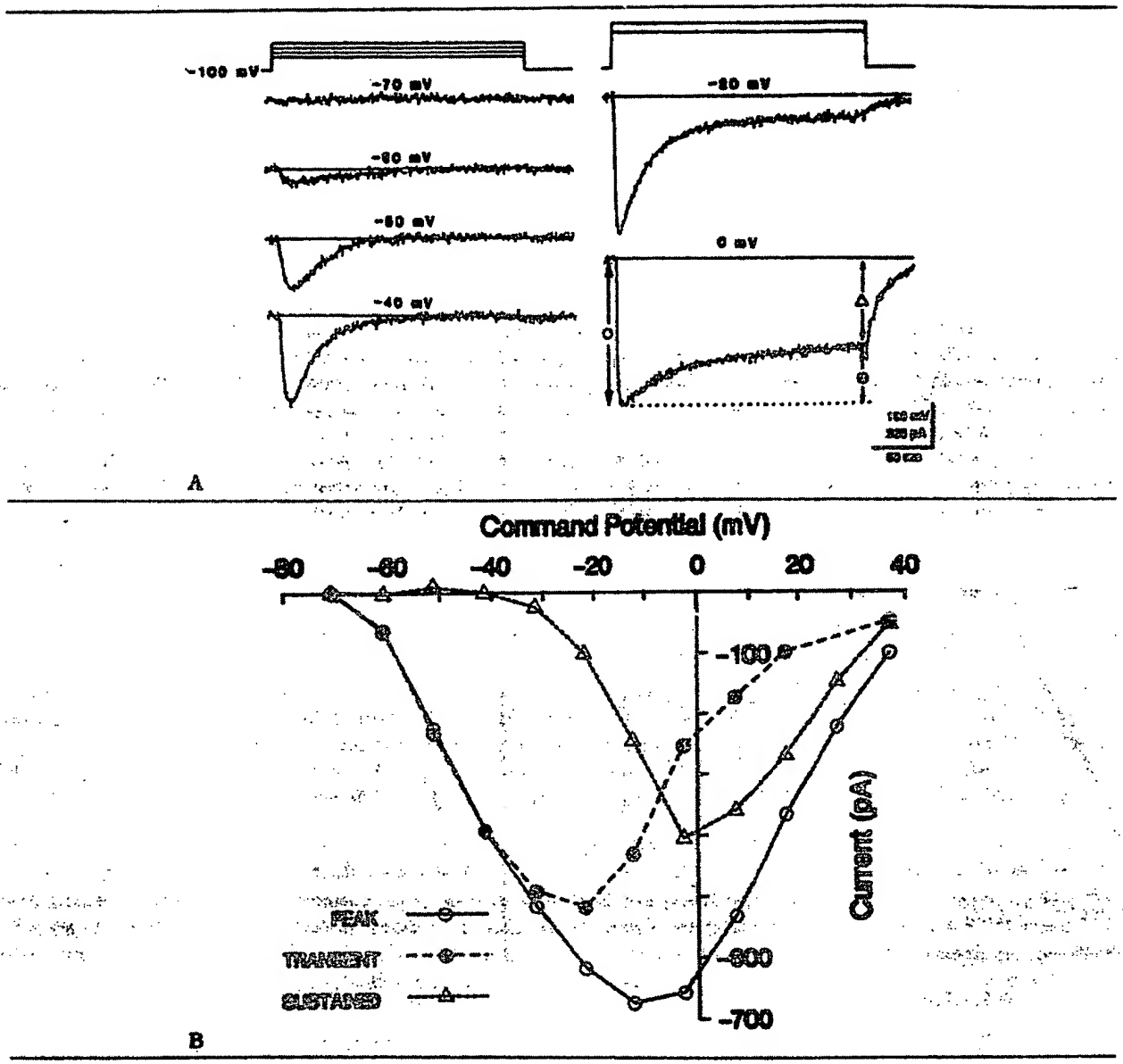

Fig 1. Calcium currents in thalamic neurons. (A) Calcium currents elicited by depolarizing voltage commands from a holding potential of −100 mV. The amplitude of the voltage command step eliciting the inward current is shown above each current trace. Note that at the most hyperpolarizing voltage commands (−60 to −40 mV) a fully inactivating, transient calcium current is evoked (left traces). *A sustained calcium current begins to appear during voltage commands to −30 mV or more depolarized levels* (right traces). *(B) Plot of calcium current from the cell illustrated in A. The peak, sustained, and transient components of calcium current (calculated as illustrated in* right bottom trace *of A) are plotted versus the voltage command eliciting the current. Note that the transient component of calcium current is evoked at a lower threshold and is equal in maximal amplitude or greater than the sustained component in the same cell. In this and all subsequent figures the command voltage eliciting the current is above the current trace, and a downward deflection is an inward current.*

others, and will be referred to as the high-threshold calcium current (HTCC). Steps to potentials more depolarizing than 0 mV resulted in a gradual decrease in the size of the HTCC, as the voltage command approached the reversal potential for the calcium current (see Fig 1, IV plot). At holding potentials of −30 mV the LTCC was inactivated, and the HTCC could be evoked in isolation by depolarizing steps (not shown). The effects of anticonvulsants on these two components of whole-cell calcium current could thus be examined by eliciting responses from a holding potential of −100 mV to step commands of −40 mV (to illustrate effects on the LTCC in isolation), and to 0 mV (where the HTCC is maximal). Both of these currents were blocked by perfusion of 500 μM Cd^{2+} (not shown).

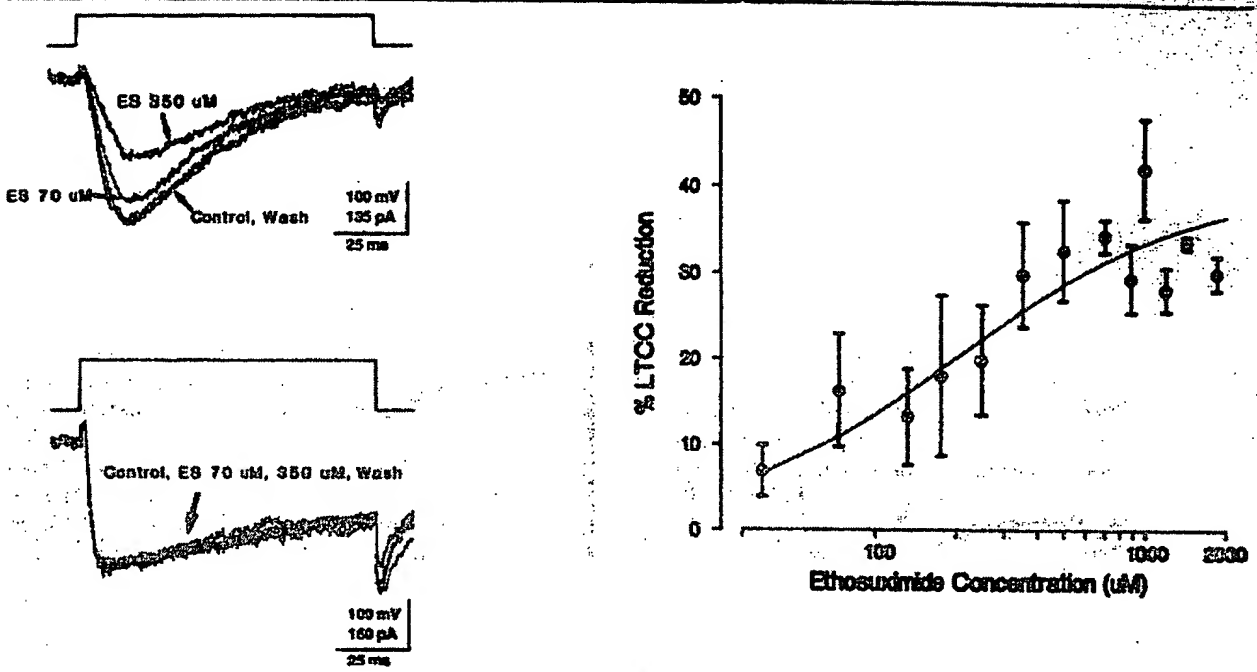

Fig 2. Ethosuximide (ES)-induced reduction of low-threshold transient calcium current (LTCC). (A) Upper sweeps: *Superimposed traces of LTCC evoked by a depolarizing voltage command to −40 mV from a holding potential of −100 mV in control solution, ES 70 μM (reducing LTCC 16%), ES 350 μM (reducing LTCC 46%), and washout of ES (complete recovery).* Lower sweeps: *Superimposed traces of high-threshold calcium current (HTCC) evoked in the same cell by a depolarizing voltage command to 0 mV from a holding potential of −100 mV, following application of ES 70 and 350 μM and washout. No effect of ethosuximide application on HTCC was visible in this neuron. (B) Plot of ES bath concentration (logarithmic scale) versus percent reduction of LTCC (at −40 mV command potential) for 23 ES-responsive thalamic neurons (error bars show mean ± SE, mean of 2–7 cells per point). The curve, which was fitted to the data by eye, is constructed from the standard dose-response equation: effect = maximal effect * ES concentration/(ES concentration + EC_{50}), using an EC_{50} estimate of 200 μM and a maximal effect estimate of 40%. This equation assumes a one-to-one stoichiometry between ES concentration and effect on calcium current.*

Ethosuximide Reductions of Calcium Currents

Applications of ethosuximide in concentrations of 50 to 1,000 μM reversibly reduced the LTCC and in some cases the HTCC (Fig 2A) in 77 of 101 neurons, as we have previously described [15]. A portion of the population (23%) was unresponsive to ethosuximide. No differences in calcium current properties between ethosuximide-unresponsive and ethosuximide-responsive neurons were detected. Ethosuximide reduction of LTCC was maximal at concentrations of 500 μM. For 10 ethosuximide-responsive cells, application of 500 μM ethosuximide resulted in a reduction of LTCC (assessed by voltage commands to −40 mV from a holding potential of −100 mV) of 31.9 ± 4% (mean ± SE). For the same cells, ethosuximide reduced the HTCC (assessed by voltage commands to 0 mV from a holding potential of −100 mV) by 2.8 ± 7.5% (cf., upper and lower traces of Fig 2A). Ethosuximide reduction of LTCC was dose dependent and could be fit by a curve generated by an equation that assumed one-to-one stoichiometry, a maximal reduction of LTCC of 40%, and an EC_{50} of 200 μM (curve in Fig 2B).

Ethosuximide reduced calcium currents over the full range of activation potentials (Fig 3), although the percentage reduction was largest at more hyperpolarized step commands and decreased with more depolarized step commands. This effect is illustrated in Figure 4A, where percentage reduction in calcium currents by ethosuximide is plotted versus step command potential for the ethosuximide application illustrated in Figure 3. This plot clearly shows that the ethosuximide effect on the LTCC is voltage dependent. This voltage dependence may explain some of the specificity of ethosuximide action on the LTCC as compared with the HTCC: the LTCC is evoked by more hyperpolarized step commands than the HTCC, and therefore would be more affected by ethosuximide. In Figure 4B, the amplitude of the ethosuximide-blocked current is plotted versus the voltage eliciting the current for the cell shown in Figures 3 and 4A. Ethosuximide reduction of calcium currents occurred only in potential ranges overlapping the activation range of the LTCC (cf., Figs 1, 3C).

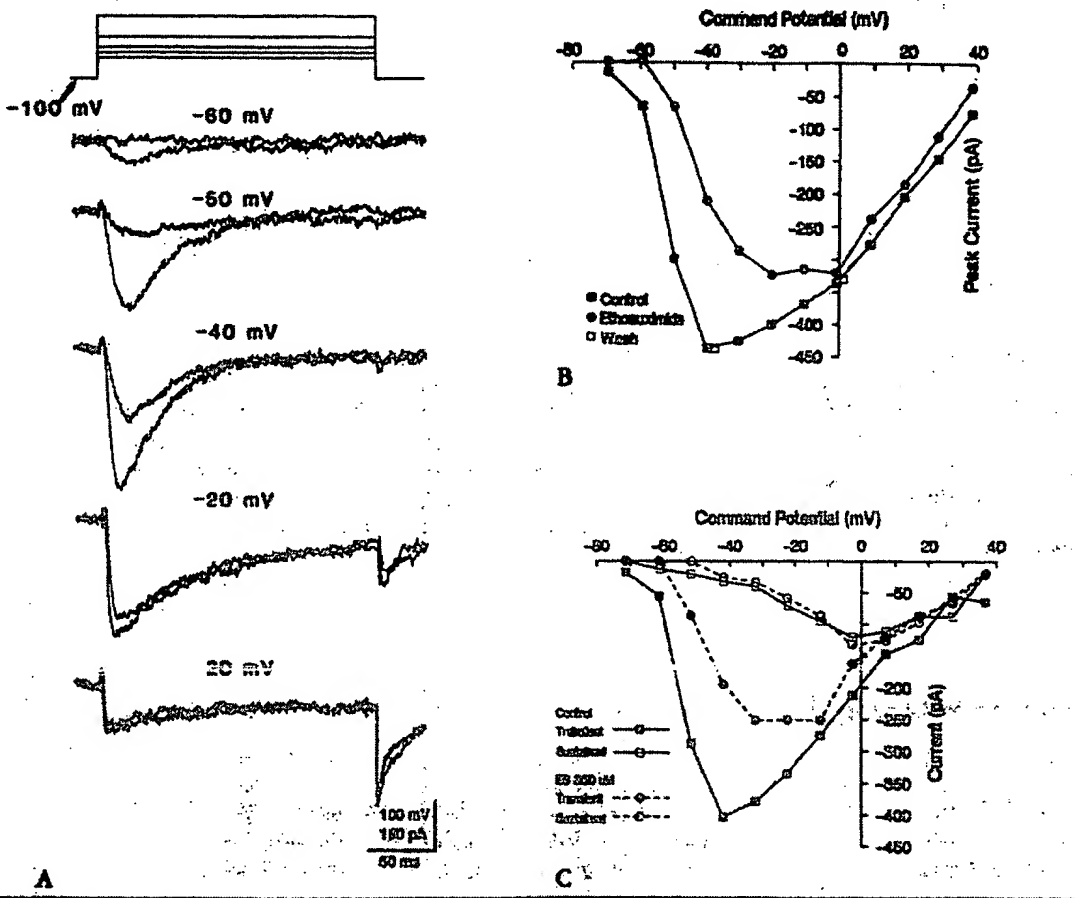

Fig 3. (A) Effect of ethosuximide (ES, 350 μM) on calcium current evoked by varying depolarizing commands from a holding potential of −100 mV. Depolarizing command level eliciting the current accompanies each trace. Two superimposed traces in each line show the control current and the current after ES application. Note that ES reduction of calcium current is maximal at more hyperpolarized command potentials. (B) Plot of voltage command amplitude versus peak calcium current under control, ES-exposed (350 μM), and wash conditions for the same cell as in A. (C) Plot of voltage command amplitude versus transient and sustained calcium current for the same cell as in A, separated as in Figure 1. Note that ES reduces calcium current in a voltage range overlapping the activation range for low-threshold calcium current (LTCC) and that this effect is voltage dependent, fully reversible, and specific for the LTCC (there is no apparent effect on the sustained component of calcium current).

Mechanism of Ethosuximide Block of Calcium Currents

The ethosuximide-induced reductions in LTCC might be produced by a change in the activation or inactivation kinetics or voltage dependence of the current, a decrease of the number of available LTCC channels, or a decrease in the single channel conductance. We performed experiments to test some of these possibilities. Records of control and ethosuximide-reduced LTCC traces were normalized so that the peak amplitudes of both traces were the same. The time course of activation and inactivation of the LTCC could then be directly compared under control and ethosuximide-exposed conditions. If ethosuximide reduced LTCC by slowing activation or speeding inactivation kinetics, a change in the time course of the current would be expected. The time course of the LTCC was identical in normalized current traces from control and drug-treated neurons (Fig 5), suggesting that ethosuximide reduces the LTCC without affecting the kinetics of activation or inactivation.

A second mechanism by which ethosuximide could reduce the LTCC might be to shift the voltage dependence of steady-state inactivation of this conductance to more hyperpolarized levels. This would reduce the number of available LTCC channels that could be activated by a given depolarizing command and consequently reduce the amplitude of the total LTCC. Figure 6 shows a comparison of steady-state inactivation curves under control and ethosuximide (350 μM)-exposed conditions (in a neuron where the drug reduced the LTCC by 46%). When the available fraction of LTCC was plotted against the holding potential, no shift in the voltage dependence of steady-state inactivation was found (see Fig 6B).

Another possibility is that ethosuximide reduces LTCC by prolonging the time course of recovery from

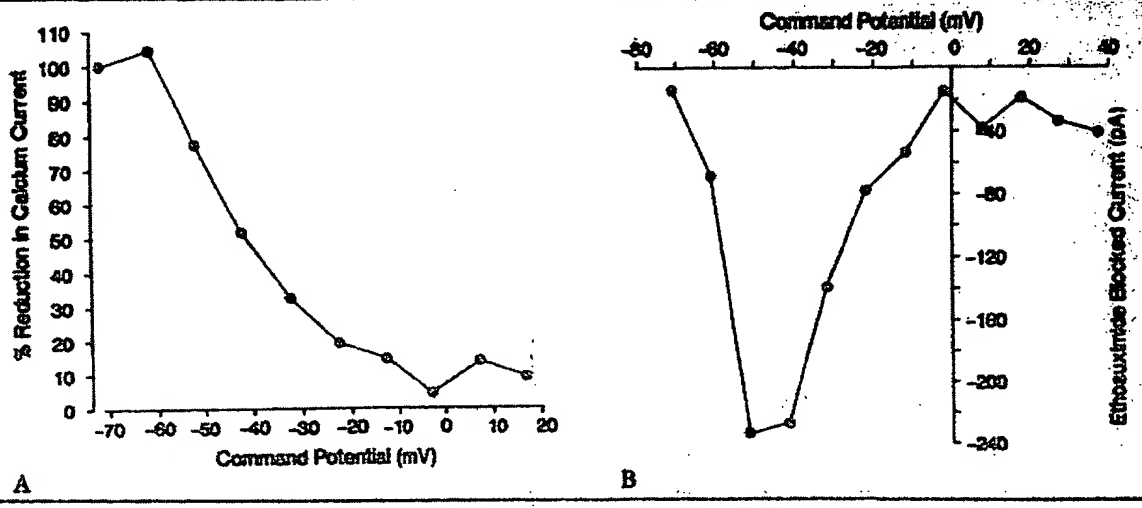

Fig 4. Voltage dependence of ethosuximide low-threshold calcium current (LTCC) reduction. (A) Plot of percent reduction of peak calcium current versus command potential eliciting the current. Note that the percentage reduction is largest at more hyperpolarized command potentials (−60 to −40 mV). (B) Plot of amplitude of ethosuximide-blocked calcium current versus command potential eliciting the current. The ethosuximide-blocked current amplitude is derived by subtracting the amplitude of calcium current under ethosuximide-exposed conditions from that under control conditions. Note that the ethosuximide-reduced current peaks at −50 to −40 mV and has a voltage activation range identical to that of the LTCC (cf., Fig 1B). Both A and B are derived from the IV plot of Figure 3B. Holding potential = −100 mV.

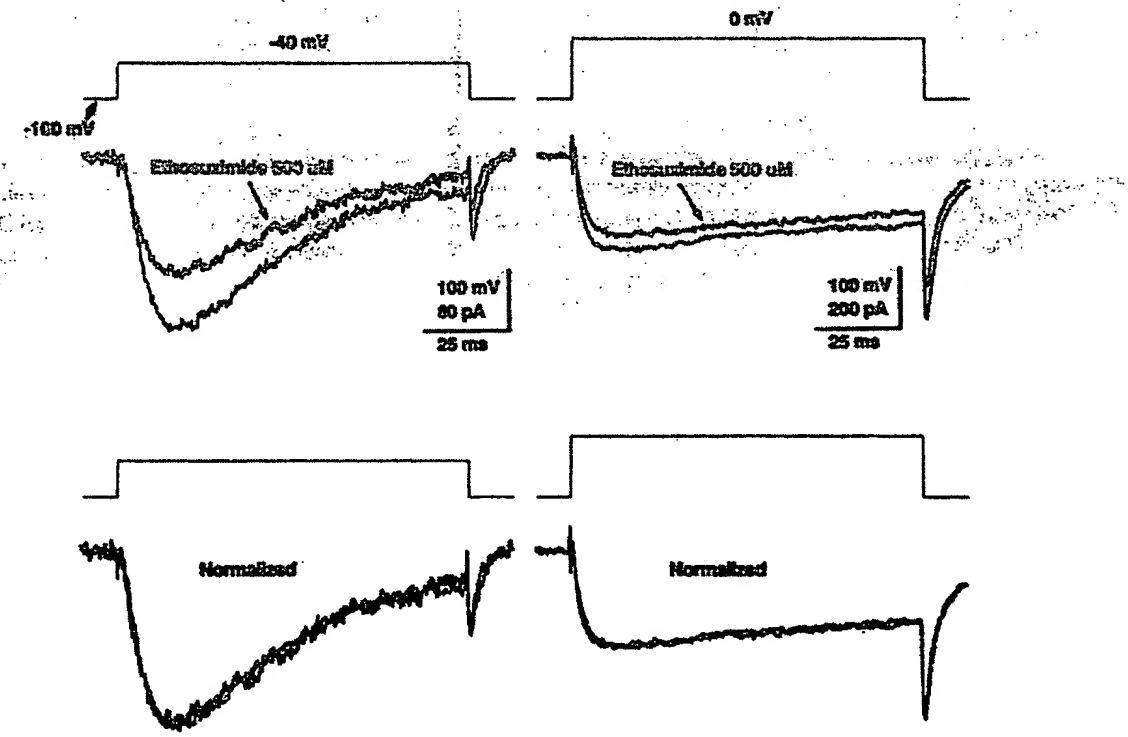

Fig 5. Ethosuximide calcium current reduction is accomplished without altering the time course of activation or inactivation of the current. Top sweeps: *Control and ethosuximide-reduced low-threshold calcium current (LTCC)* (left) *and high-threshold calcium current (HTCC)* (right) *used to obtain normalized traces.* Bottom sweeps: *Superimposed traces of control and ethosuximide-reduced calcium currents normalized to peak amplitude. Note that the ethosuximide-reduced LTCC* (left traces, *32% reduction) and HTCC* (right traces, *15% reduction) have time courses that are identical to control currents (i.e., normalized current traces overlap almost completely). Ethosuximide concentration was 500 μM. Voltage commands and holding potential are the same in bottom and top sweeps of each column.*

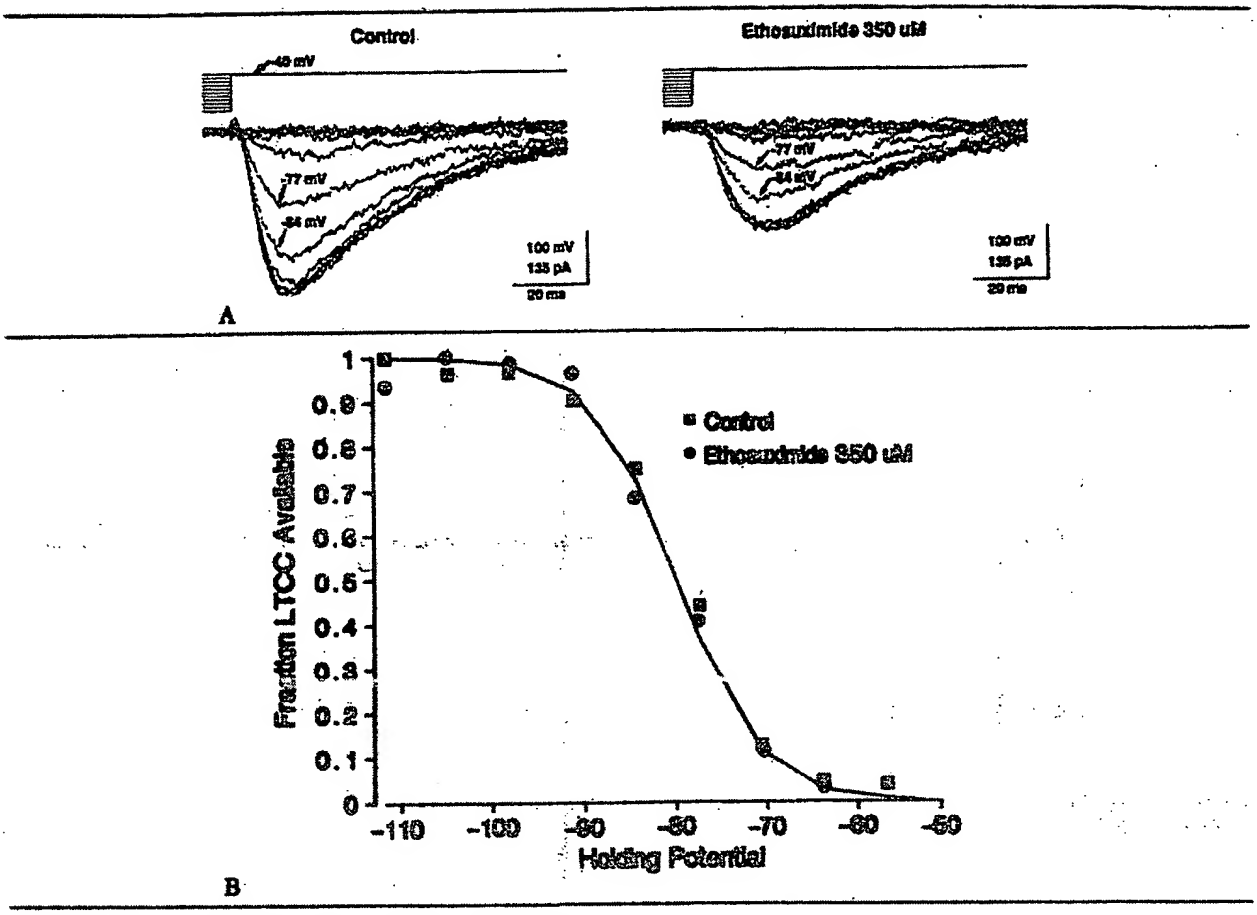

Fig 6. Steady-state inactivation of control and ethosuximide-reduced low-threshold calcium current (LTCC). Top: *Superimposed sweeps of control* (left) *and ethosuximide-reduced* (right) *LTCC evoked by depolarizing commands to −40 mV from varying holding potentials* (upper traces). *Note the dependence of LTCC amplitude (but not time course) on holding potential.* Bottom: *Plot of holding potential versus fraction of LTCC available (relative to LTCC evoked from −112 mV) derived from the superimposed traces above. Note that ethosuximide has no effect on the voltage dependence of steady-state inactivation. Records are from a cell where 350 μM ethosuximide reduced LTCC by 46% in a completely reversible manner.*

inactivation to such an extent that subsequent voltage steps find the neuron still recovering from the previous depolarization and a portion of the LTCC channels unavailable for activation. This explanation for the ethosuximide effects on LTCC was also inadequate, since the time course of recovery from inactivation under control and ethosuximide-exposed conditions was similar (Fig 7) in a cell where ethosuximide (500 μM) reduced the LTCC by 30%.

Dimethadione Reductions of Calcium Currents

Trimethadione was a frequently prescribed specific petit mal anticonvulsant prior to the development of ethosuximide and valproic acid. In the body, it is *N*-demethylated to dimethadione, the active metabolite, which is only poorly metabolized and accumulates in high concentrations (therapeutic free serum levels are 5–9 mM) [19, 20]. When applied to acutely isolated thalamic neurons in concentrations of 4 or 8 mM, dimethadione reversibly reduced both the LTCC and HTCC (LTCC reduction 52.4 ± 3.6%, HTCC reduction 34 ± 5.8%, concentration 8 mM, n = 10; LTCC reduction 40.0 ± 5.5%, HTCC reduction 31.2 ± 4.5%, concentration 4 mM, n = 5) (Fig 8). Like ethosuximide, dimethadione caused a reduction of LTCC over the full range of activation voltages (see Fig 8A,B). Dimethadione-reduced calcium currents showed no changes in their time course of activation or inactivation when compared with control currents (see Fig 8A). The reductions of LTCC showed similar voltage dependence to those produced by ethosuximide (see Fig 8C). Dimethadione also had no effect on the voltage dependence of steady-state inactivation (Fig 8D) or on the time course of recovery from inactivation (not shown). These findings suggest that dimethadione may reduce calcium currents in thalamic neurons through a mechanism similar to ethosuximide's. Structural parallels between these two molecules may be related to their similar actions on calcium currents (Fig 9).

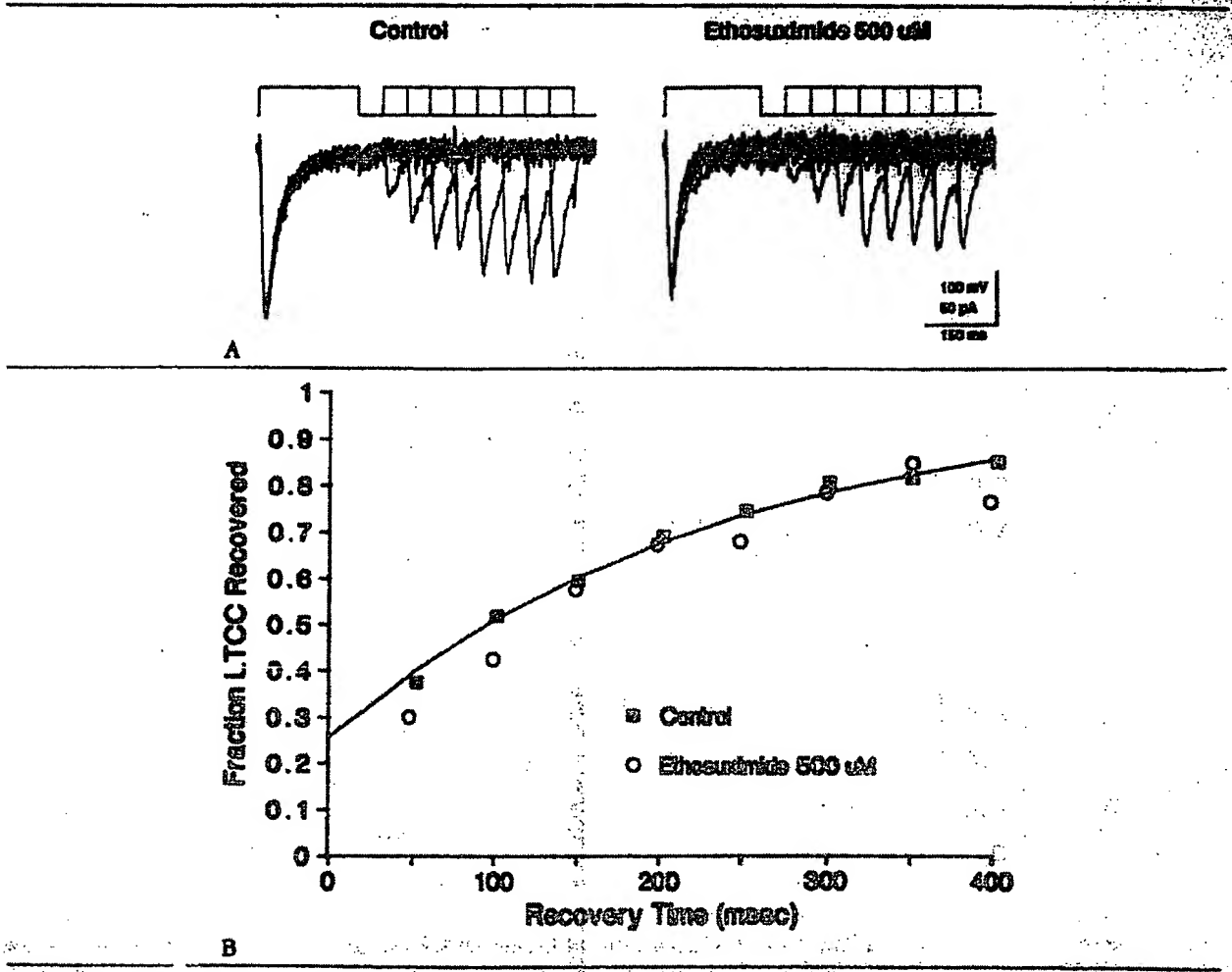

Fig 7. Recovery from inactivation of control and ethosuximide-reduced low-threshold calcium current (LTCC). Top: *superimposed traces illustrating the time course of recovery from inactivation of control* (left) *and ethosuximide-reduced* (right) *LTCC. The LTCC was first inactivated by a 200 msec depolarizing command to −40 mV from a holding potential of −100 mV. At varying times after the offset of this command, the degree of recovery of the LTCC was assessed by a 50-msec depolarizing command to 40 mV.* Bottom: *Plot of recovery time versus fraction of LTCC recovered for control and ethosuximide-reduced LTCC derived from the top traces. Note that ethosuximide has little effect on the time course of recovery from inactivation. Records are from a cell where ethosuximide (500 μM) reduced LTCC by 30% in a completely reversible manner.*

Other Anticonvulsants

Valproic acid, another anticonvulsant effective against petit mal, is structurally and functionally quite different from ethosuximide and dimethadione (see Fig 9). It has a very broad spectrum of action, including effectiveness in control of other types of generalized seizures [25]. When applied to thalamic neurons, valproic acid had no effect on calcium currents at concentrations up to 1 mM (n = 12). Phenytoin, a drug effective in partial or generalized motor seizures, but ineffective in the control of petit mal, shares some structural features with ethosuximide (Fig 9). These structural similarities, and reports that phenytoin reduces LTCC in cultured hippocampal neurons [26] and neuroblastoma cells [27], prompted us to examine the effects of this agent on calcium currents in thalamic cells. At concentrations of 4 to 8 μM (therapeutic free serum levels), this drug had only small effects on calcium currents (less than 10% reductions of the LTCC, n = 8). At 100 μM, a concentration that far exceeds clinically relevant levels, phenytoin reduced the LTCC by 44 ± 2.8% and the HTCC by 3.3 ± 2.3% (n = 6) (Fig 10).

Discussion

We have previously reported that ethosuximide reduces the LTCC in enzymatically dissociated thalamic neurons [15] in a dose-dependent manner at concentrations very similar to the free serum levels achieved in petit mal patients whose seizures were controlled [2]. Our present results provide additional information about the nature of this reduction of LTCC by ethosuximide. Ethosuximide effects occurred without

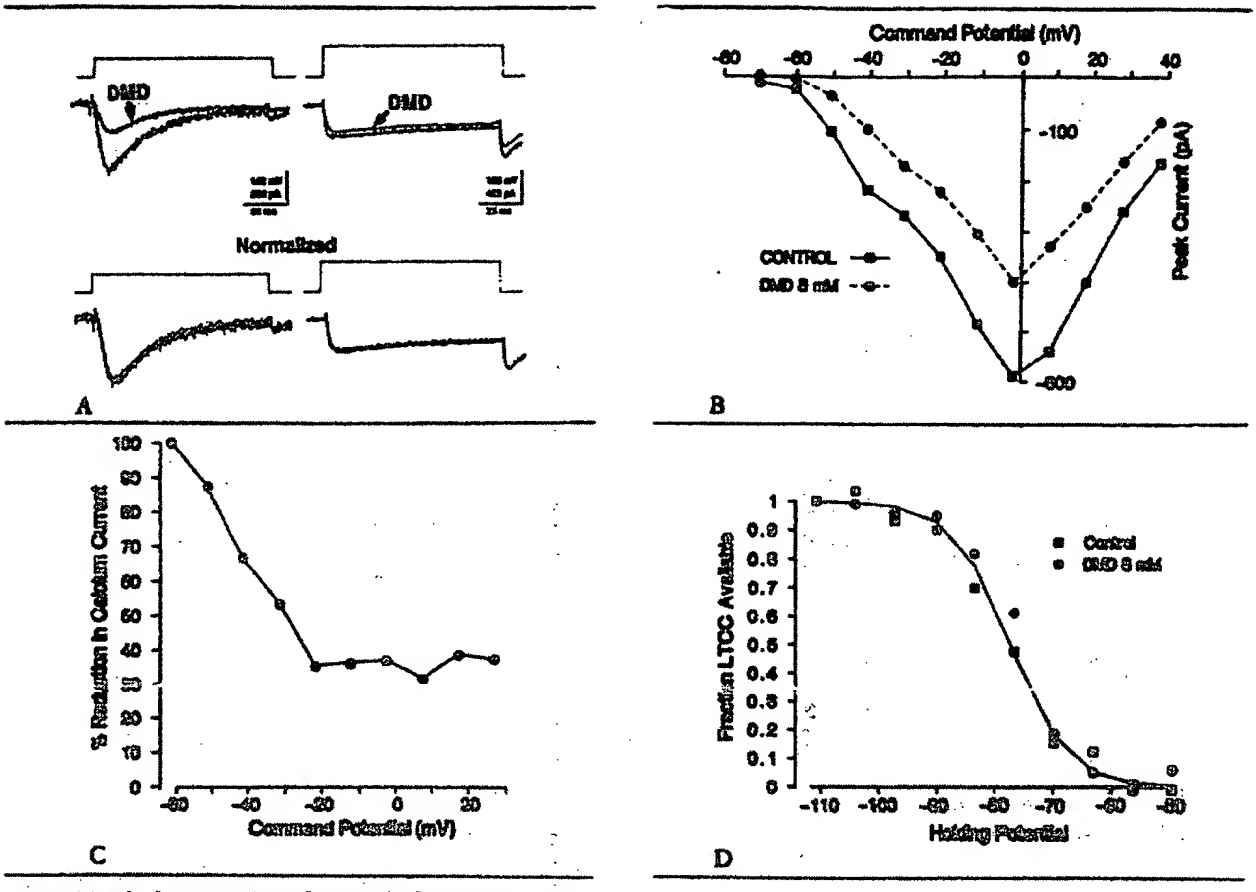

Fig 8. Dimethadione (DMD) reduction of calcium currents. (A) DMD reduces low (LTCC) and high-threshold calcium currents (HTCC) without altering the time course of activation or inactivation of either current. Top traces: *Control and DMD-reduced LTCC* (left, *55% reduction) and HTCC* (right, *19% reduction).* Bottom traces: *Superimposed normalized traces of control and DMD-reduced LTCC* (left) *and HTCC* (right). *Note that the drug-reduced and control traces have identical time courses. (B) IV plot of calcium currents under control and DMD-exposed conditions. Voltage command potential (from a holding potential of −100 mV) is plotted versus the peak amplitude of the elicited calcium current. Note that DMD reduces calcium current over the full range of activation potentials. (C) Plot of percent reduction of calcium current by DMD versus the command potential eliciting the current for the IV plot in B. Note that the DMD reduction of calcium current is voltage dependent. (D) Steady-state inactivation of control and DMD-reduced LTCC, derived as in Figure 6 for ethosuximide. Plot of holding potential versus fraction of LTCC available (relative to LTCC evoked from −112 mV). Note that DMD has no effect on the voltage dependence of steady-state inactivation. Records are from a cell where 9 mM DMD reduced LTCC by 55% in a completely reversible manner.*

changes in the time course of activation or inactivation of the LTCC and were evident over the entire voltage activation range. Further, reductions in LTCC occurred without alterations in the voltage dependence of activation or steady-state inactivation, or the time dependence of recovery from inactivation. Taken together, these data suggest that ethosuximide does not reduce the LTCC by exerting direct effects on channel kinetics or gating mechanisms. Rather, the results are compatible with a decrease in the number of available LTCC channels or in the elementary single LTCC channel conductance. Direct studies of LTCC channels will be required to confirm this conclusion. Dimethadione appeared to reduce LTCC by a similar voltage-dependent mechanism, but was less specific in that it significantly reduced the HTCC as well as the LTCC.

Twenty-three percent of the thalamic neurons studied did not respond to any concentration of ethosuximide with a reduction in calcium current, even though they possessed an LTCC that was indistinguishable from that in other, responsive neurons. This finding appears inconsistent with the LTCC channel–blocking hypothesis: ethosuximide channel–blocking actions would be expected to occur in all cells with low-threshold calcium channels. It is possible that the enzymatic treatment in some way altered the nonre-

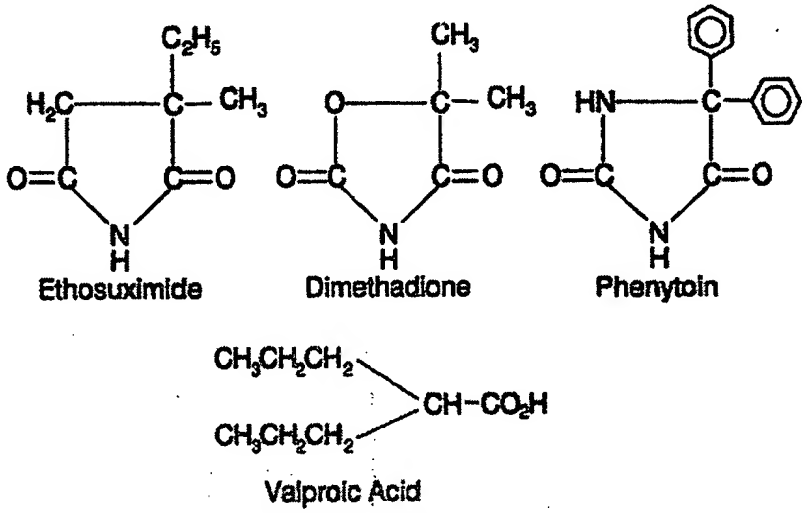

Fig 9. Structures of the anticonvulsants whose actions on calcium currents were examined in this study. Note the similar ring structures and substitutions of ethosuximide, dimethadione, and phenytoin, and the dissimilar structure of valproic acid.

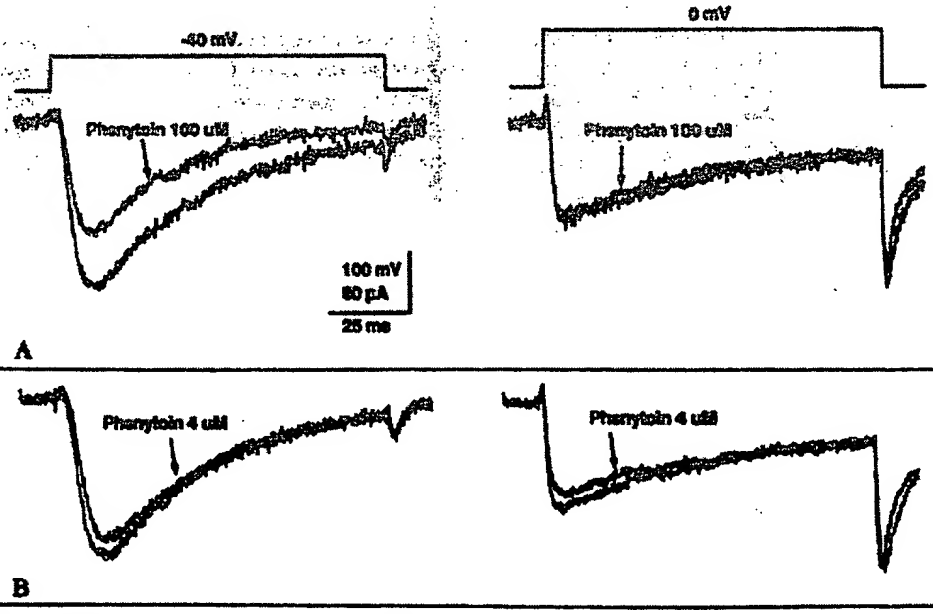

Fig 10. Phenytoin reductions of calcium currents. (A) At high concentration (100 μM), phenytoin specifically reduced low-threshold calcium current (LTCC) by 33% (left) without having an effect on the high-threshold calcium current (right). (B) In the same cell, following washout of 100 μM phenytoin, low, clinically relevant concentrations of phenytoin (4 μM) had only small effects on LTCC (< 10% reduction, left). Control and drug sweeps are superimposed.

sponsive cells so that they could not bind ethosuximide. By analogy with the actions of dihydropyridines on sustained calcium current in other cells [28], the receptor for ethosuximide could be the low-threshold calcium channel itself, somehow modified in a subset of the cells so as to be inaccessible to ethosuximide. Alternatively, there may be subsets of relay neurons that differ in their pharmacological sensitivity to these agents.

Consideration of the normal role of LTCC in the behavior of thalamic neurons may provide insights about the mechanisms by which ethosuximide-induced depression of this current could lead to control of spike-wave discharges. In contrast to its size in other mammalian neurons [18, 22, 24], the LTCC is particularly prominent in thalamic cells, where its overall peak amplitude is usually equal to or greater than that of the HTCC (e.g., Figs 1, 4C, and [21]). This proportionally large LTCC influences the behavior of thalamic neurons, as seen in current-clamp recordings, where large, low-threshold calcium-dependent spikes (LTCSs) are a prominent feature of cell behavior [10–13]. Given the kinetics of its voltage- and time-dependence of activation, inactivation, and deinactivation (see Figs 1–7) [11, 17, 22, 24], the LTCC is the only calcium conductance that could be responsible for the LTCSs. The LTCS is particularly important in regulating the normal oscillatory behavior of thalamic neurons [10, 12–14]. Normal afferent barrage, as occurs in the waking state, will maintain the resting membrane potential at a depolarized level sufficient to inactivate the LTCS, so that simple relay behavior without bursting predominates. Under conditions where the membrane potential is hyperpolarized, either by reduction of afferent drive (as in quiet sleep) or by strong hyperpolarizing inputs (e.g., [13]), the LTCS will be deinactivated and tend to promote bursting. Sequential activation of the LTCC and repolarizing potassium conductances can produce intrinsic oscillatory behavior [10–12]. These properties, together with the tight reciprocal connectivity between the thalamus and cortex, predispose the network to thalamocortical oscillations (reviewed in [14]) such as occur during sleep spindles.

Although direct evidence is not available in animal models of petit mal, or in humans, the above considerations make it likely that the LTCS and underlying LTCC play in important role in generating the abnormal rhythmicity that characterizes spike-wave discharges. This conclusion is further supported by the close relationship between spindles and spike waves present both in the feline penicillin model of generalized spike-wave discharge [6] and in human petit mal during spindle stage sleep (see Kellaway [29] for review) when spike-wave discharges are most prominent. Synchronous corticothalamic volleys such as occur during experimental spike-wave discharges [5, 7] can activate the LTCS in a postsynaptic population (e.g., [30]), with resulting spike burst generation in thalamic neurons that would propagate over thalamocortical axons and reactivate the cortical population. Even small reductions in the LTCS with ethosuximide would tend to dampen or abolish this type of oscillation and hence reduce spike-wave discharges and absence seizures. These effects would be significantly enhanced by the voltage dependence of the ethosuximide-induced LTCC reduction (see Fig 4), which would cause threshold calcium conductances, evoked by small depolarizations from hyperpolarized potentials, to be particularly affected. Thus, the action of ethosuximide on LTCC of thalamic neurons seems consistent with its anticonvulsant action in petit mal seizures, as does its ability to reduce thalamocortical transmission selectively at 3 to 4 Hz in vivo in cats [31, 32].

Dimethadione reduced the LTCC to a greater extent than did ethosuximide (maximal LTCC reductions, assessed during a voltage command to −40 mV, were 52.4% versus 31.9% for dimethadione and ethosuximide, respectively), although both drugs appeared to act through similar mechanisms (cf., Figs 3–6, 8). This greater efficacy of dimethadione appears to be at odds with the relative clinical effectiveness of these two agents as anticonvulsants. Because of their voltage-dependent actions, however, these two agents were equally effective in reducing calcium currents at threshold potentials (cf., Figs 4A, 8C). Phenytoin, which is also structurally similar to ethosuximide (see Fig 9), reduces LTCC only at toxic concentrations (see Fig 10), and so its actions on LTCC are most likely unrelated to its anticonvulsant action.

Not all petit mal anticonvulsants share the action of ethosuximide on calcium currents in thalamic neurons. Valproic acid had no effect on LTCC in concentrations up to 1 mM. Other effects of valproate [8, 33, 34] may be responsible for its diverse anticonvulsant actions. It is also conceivable that valproate metabolites not tested in our acute experiments (e.g., 2-en-valproate, 3-keto-valproate, or various hydroxy-valproates; see Löscher and colleagues [35]) have effects on the LTCC.

The finding that specific petit mal anticonvulsants reduce LTCC in thalamic neurons does not necessarily imply that abnormalities of LTCC regulation or function play a role in the pathogenesis of petit mal epilepsy, although this will certainly be an interesting hypothesis to explore. This result does suggest, however, that the LTCC is important in the generation and maintenance of spike-wave discharges underlying petit mal seizures and that modulation of the properties of the LTCC could influence petit mal attacks. The well-known effects of the level of arousal on both petit mal seizures [29] and thalamic LTCSs [14] may be an example of such modulatory influences. We are not implying that reductions in the LTCC are the only mech-

anism of action of ethosuximide, since other possible effects in the thalamus or at other sites have not been examined in this study (but see [3] for review). The important finding is an effect of ethosuximide that is consistent with its anticonvulsant action. The LTCC reduction occurs in clinically relevant drug concentration ranges and is shared by another specific petit mal anticonvulsant (dimethadione). The effects of these compounds on the LTCC are not mimicked by succinimide [15], a close structural relative that is inactive as an anticonvulsant [3]. Thus, LTCC reduction appears to be closely related to specific petit mal anticonvulsant effectiveness.

Supported by NIH grants NS06477, NS12151, and NS07280 and the Morris and Pimley research funds.

We thank H. Feeser for technical assistance.

References

1. Aicardi J. Epilepsy in children. New York: Raven, 1986
2. Browne TR, Dreifuss FE, Dyken PR, et al. Ethosuximide in the treatment of absence (petit mal) seizures. Neurology 1975;25:515–524
3. Ferrendelli JA, Kupferberg HJ. Antiepileptic drugs. Succinimides. In: Glaser GH, Penry JK, Woodbury DM, eds. Antiepileptic drugs: mechanisms of action. New York: Raven, 1980
4. Williams D. A study of thalamic and cortical rhythms in petit mal. Brain 1953;76:50–69
5. Avoli M, Gloor P, Kostopoulos G, Gotman J. An analysis of penicillin-induced generalized spike and wave discharges using simultaneous recordings of cortical and thalamic single neurons. J Neurophysiol 1983;50:819–837
6. McLachlan RS, Avoli M, Gloor P. Transition from spindles to generalized spike and wave discharges in the cat: simultaneous single-cell recordings in cortex and thalamus. Exp Neurol 1984;85:413–425
7. Vergnes M, Marescaux C, Depaulis A, et al. Spontaneous spike and wave discharges in thalamus and cortex in a rat model of genetic petit mal–like seizures. Exp Neurol 1987;96:127–136
8. McLean MJ, Macdonald RL. Sodium valproate, but not ethosuximide, produces use- and voltage-dependent limitation of high frequency repetitive firing of action potentials of mouse central neurons in cell culture. J Pharmacol Exp Ther 1986;237:1001–1011
9. Fohlmeister JF, Adelman WJ Jr, Brennan JJ. Excitable channel currents and gating times in the presence of anticonvulsants ethosuximide and valproate. J Pharmacol Exp Ther 1984;230:75–81
10. Deschênes M, Paradis M, Roy JP, Steriade M. Electrophysiology of neurons of lateral thalamic nuclei in cat: resting properties and burst discharges. J Neurophysiol 1984;51:1196–1219
11. Jahnsen H, Llinás R. Electrophysiological properties of guinea-pig thalamic neurones: an *in vitro* study. J Physiol (Lond) 1984;349:205–226
12. Jahnsen H, Llinás R. Ionic basis for the electroresponsiveness and oscillatory properties of guinea-pig thalamic neurons *in vitro*. J Physiol (Lond) 1984;349:227–247
13. McCormick DA, Prince DA. Acetylcholine induces burst firing in thalamic reticular neurones by activating a potassium conductance. Nature 1986;319:402–405
14. Steriade M, Llinás RR. The functional states of the thalamus and the associated neuronal interplay. Physiol Rev 1988;68:649–742
15. Coulter DA, Huguenard JR, Prince DA. Specific petit mal anticonvulsants reduce calcium currents in thalamic neurons. Neurosci Lett 1989 (in press)
16. Kay AR, Wong RKS. Isolation of neurons suitable for patch-clamping from adult mammalian nervous systems. J Neurosci Methods 1986;16:227–238
17. Hamill OP, Marty A, Neher E, et al. Improved patch-clamp techniques for high-resolution current recordings from cells and cell-free membrane patches. Pflügers Arch 1981;391:85–100
18. Kay AR, Wong RKS. Calcium current activation kinetics in isolated pyramidal neurones of the CA1 region of the mature guinea-pig hippocampus. J Physiol (Lond) 1987;392:603–616
19. Booker HE. Trimethadione. Relation of plasma concentration to seizure control. In: Woodbury DM, Penry JK, Pippenger CE, eds. Antiepileptic drugs. New York: Raven, 1982
20. Chamberlain H, Waddell W, Butler T. A study of the product of demethylation of trimethadione in the control of petit mal epilepsy. Neurology 1965;8:106–112
21. Coulter DA, Huguenard JR, Prince DA. Calcium currents in thalamic relay neurones: Kinetic properties of transient, low-threshold current. J Physiol (Lond) 1989 (in press)
22. Fox AP, Nowycky MC, Tsien RW. Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurones. J Physiol (Lond) 1987;394:149–172
23. Narahashi T, Tsunoo A, Yoshi-i M. Characterization of two types of calcium channels in mouse neuroblastoma cells. J Physiol (Lond) 1987;383:231–249
24. Carbone E, Lux HD. A low voltage–activated, fully inactivating Ca channel in vertebrate sensory neurones. Nature 1984;310:501–502
25. Pinder RM, Brogden RN, Speight TM, Avery GS. Sodium valproate: a review of its pharmacological and therapeutic efficacy in epilepsy. Drugs 1977;13:81–123
26. Yaari Y, Hamon B, Lux HD. Development of two types of calcium channels in cultured mammalian hippocampal neurons. Science 1987;235:680–682
27. Twombly DA, Yoshii M, Narahashi T. Mechanisms of calcium channel block by phenytoin. J Pharmacol Exp Ther 1988;246:189–195
28. Flockerzi V, Oeken HJ, Hofmann F, et al. Purified dihydropyridine-binding site from skeletal muscle t-tubules is a functional calcium channel. Nature 1986;323:66–68
29. Kellaway P. Sleep and epilepsy. Epilepsia 1985;26:S15–S30
30. Andersen P, Eccles JC, Sears TA. The ventro-basal complex of the thalamus: types of cells, their responses, and their functional organization. J Physiol (Lond) 1964;174:370–399
31. Englander RN, Johnson RN, Brickley JJ, Hanna GR. Effects of antiepileptic drugs on thalamocortical excitability. Neurology 1977;27:1134–1139
32. Nowack WJ, Johnson RN, Englander RN, Hanna GR. Effects of valproate and ethosuximide on thalamocortical excitability. Neurology 1979;29:96–99
33. Macdonald RL, Bergey GK. Valproic acid augments GABA-mediated postsynaptic inhibition in cultured mammalian neurons. Brain Res 1979;170:558–562
34. Macdonald RL, McLean MJ. Anticonvulsant drugs: mechanisms of action. Adv Neurol 1986;44:713–736
35. Löscher W, Nau H, Siemes H. Penetration of valproate and its active metabolites into cerebrospinal fluid of children with epilepsy. Epilepsia 1988;29:311–316

79.1

A. McMahon[1]*, E. Perez-Reyes[2] and R.H. Joho[1]. [1]The Center for Basic Neuroscience, The University of Texas Southwestern Medical Center, Dallas, TX 75235, and [2]Department of Physiology, Loyola University Medical Center, Maywood, IL 60153.

GENE STRUCTURE INCLUDING UPSTREAM SEQUENCES OF THE MOUSE α1G T-TYPE CALCIUM CHANNEL.

Three genes coding for low voltage-activated calcium channels have been identified. *In situ* hybridization analysis identified α1G as the most abundantly and widely expressed of these genes in the brain. We have isolated mouse genomic clones containing promoter sequence and exon sequences spanning the first domain of α1G (from a mouse genomic library screened with either a probe containing the 5' untranslated region or coding sequence from domain 1 of the rat cDNA). Similar to other members of the calcium channel family the coding sequence for the α1G gene is distributed among many exons with general conservation of intron/exon boundaries observed between the human and mouse genes. The sequence for domain 1 is contained in 7 exons with exon sizes from less than 100 bp to 784 bp for exon 7 which encodes part of the S6 transmembrane segment and part of the cytoplasmic loop connecting to domain 2. Exon 1 contained a large untranslated sequence (almost 500 bp) followed by the sequence encoding the first 81 amino acids of the cytoplasmic domain up to the beginning of the first transmembrane segment (S1). The α1G gene promoter does not contain a TATA box but contains a very G+C rich region around the transcription start site. The promoter contains two GC boxes and two copies of the core motif CCAGGAG found in several neuron-specific genes. One of these sequences is present in inverse orientation within the sequence for the 5' untranslated region of the cDNA. The promoter sequence also contains motifs for CREB and AP-2 transcription factor-binding sites (supported by NS28407 [RHJ]).

79.2

E. McRory[1]*, J. Mezeyova[2], C.M. Santi[1], K. Hamming[1], K. Sutton[1], and T.P. Snutch[1,2]. [1]Biotechnology Lab, University of British Columbia, Vancouver B.C., Canada V6T 1Z3; [2]NeuroMed Technologies, Vancouver B.C., Canada V5Z 4C2.

ISOLATION AND CHARACTERIZATION OF A FAMILY OF T-TYPE CALCIUM CHANNELS.

Voltage gated calcium channels are encoded by a family of structurally related α1 subunit proteins. Utilizing a genomics-based cloning strategy, we have isolated three members of the T-type class of calcium channels from human and rat brain. The full length rat α_{1G} cDNA encodes a protein of 2286 amino acids, the rat α_{1H} cDNA encodes a protein of 2044 amino acids and the rat α_{1I} cDNA encodes a protein of 1799 amino acids. The α_{1G} and α_{1H} channels share 65% amino acid identity while the α_{1I} is 53% identical to α_{1H} and 47% to α_{1G}. Northern blot and RT/PCR analysis shows that α_{1H} and α_{1G} exhibit a similar expression pattern in rat brain. However, in contrast to a previous report (Talley *et al.*, J Neurosci 19(6):1895-911), we find that α_{1I} channel expression exhibits an unique and selective distribution. Several alternatively spliced variants were also found, including α_{1I} variants that differ by the presence or absence of 6 amino acids in the domain I-II linker or by the presence or absence of 13 amino acids in the domain IV S4-S5 segment. Transient expression studies in HEK 293 cells shows that each of the α_{1G}, α_{1H} and α_{1I} subunits encode functional T-type calcium channels.

Supported by grants from MRC of Canada, NSERC of Canada, ALS Society of Canada and the Human Frontiers Science Foundation.

79.3

S. Mittman*, J. Guo and W.S. Agnew. Depts. of Anesthesiology and Physiology, The Johns Hopkins University School of Medicine, Baltimore, MD 21287

STRUCTURE AND ALTERNATIVE SPLICING OF THE GENES ENCODING THE HUMAN BRAIN T Ca^{2+} CHANNEL SUBUNITS α_{1G} AND α_{1I}.

The structures of the genes encoding the human brain T Ca^{2+} channel subunits α_{1G} and α_{1I} (*CACNA1G* and *CACNA1I*) were determined by comparison of genomic and PCR-amplified brain cDNA sequences. *CACNA1G* consists of ≥38 exons spanning ≥66,490 nt of chromosome 17q22. Alternative splicing of the RNA occurs at six sites: cassette exons 14, 26, 34 and 35, an internal donor in exon 25 and protein-coding intron 38B. Additionally, the RNA can be polyadenylated at either of two sites. *CACNA1I* consists of ≥37 exons spanning ≥116,390 nt of chromosome 22q12.3-13.2. Alternative splicing of the RNA occurs at three sites: cassette exon 9, an internal acceptor in exon 33 and an internal donor in exon 36. Polyadenylation and cleavage at the 3′ end of the long version of exon 36 leads to loss of exon 37. Alternative processing of the *CANCA1G* and *CACNA1I* RNAs may lead to expression of as many as 64 and 8 distinct protein products, respectively. This molecular diversity may contribute to the observed heterogeneity of T currents in central neurons. Supported by the NIH, the Foundation for Anesthesia Education and Research, the American Society of Regional Anesthesia and Astra Pharmaceutical Products, Inc.

79.4

M. Monteil, J. Chemin, S. Spiesser, E. Bourinet*, P. Lory, and J. Nargeot. Institut de Génétique Humaine, CNRS UPR1142, 141 Rue de la Cardonille, 34396 Montpellier France.

IDENTIFICATION OF HUMAN α_{1G} T-TYPE CALCIUM CHANNEL SPLICE VARIANTS.

Voltage Gated Calcium Channel (VGCC) diversity is abundantly described in native neurons. They are classified mainly on the basis of their sensitivity to membrane depolarization (Low Threshold of Activation or LVA *vs* High Threshold of Activation or HVA) and their distinct pharmacological properties. Different functions have been linked to these various channels ranging from cellular excitability to neurotransmitter release or gene activation. Molecular cloning of the constitutive subunits of these channels progressively shows the extent of their structural diversity. On this aspect, HVA VGCC have been well characterized. They are multimeric complexes formed of an α1 pore forming subunit (SU) and at least 3 ancillary SU (β, α2-δ, and γ). To date seven genes encoding HVA α1 SU are identified (α_{1A}, α_{1B}, α_{1C}, α_{1D}, α_{1E}, α_{1F}, α_{1S}). Recently, 3 novel α1 SU have been identified[(1)] and recognized as major components of LVA (or T-type) Ca2+ channel (α_{1G}, α_{1H}, and α_{1I}). We have cloned and expressed several isoforms of human α_{1G} subunit. The functional properties of these splice variants have been studied following expression in HEK cells. Tissues such as cerebellar and thalamic neurons or developping heart myocytes where an implication of T-type Ca2+ channel has been postulated in the genesis of rhythmic oscillations of the membrane potential shows high levels of α_{1G} expression. Therefore additional characterization of α_{1G} isoforms activity have been done using action potential waveforms of these different cells as voltage command to investigate how their respective properties are compatible with a role on pacemaking activity.

(1) Perez-Reyes et al (1998) Nature 391 : 896-899 ; Lee J-H. et al (1999) J Neurosci 19 : 1912-1921.

79.5

C.M. Santi[1], J.E. McRory[1], K.G. Sutton[1], J. Mezeyova[2], A. Hasson[1]* and T.P. Snutch[1]. [1]Biotechnology Laboratory, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1Z3; [2]NeuroMed Technologies Inc.,Vancouver, B.C., Canada, V5Z 4C2.

α_{1I}: A T-TYPE CALCIUM CHANNEL WITH NOVEL GATING PROPERTIES.

Low voltage activated calcium channels play an important role in the generation of pacemaker activity and repetitive bursting in neurons. Here we present a detailed analysis of the electrophysiological properties of a new member of the T-type calcium channel family: α_{1I}. We compare the properties of α_{1I} with another member of the low-threshold calcium channel family (α_{1G}) and the α_{1E} channel which exhibits properties of both high- and low-threshold channels. Channels were transiently expressed in HEK 293 cells and currents recorded in 2 mM calcium external solution using the whole cell patch clamp technique. The α_{1I} channel both activated (~ -80 mV) and peaked (~ –50 mV) at more negative potentials compared with α_{1G} and α_{1E}. The V_{50act} for α_{1I} was found to be -60.7 mV compared to -51.6 mV and -9.2 mV for α_{1G} and α_{1E}, respectively. The α_{1I} channel also exhibited relatively more negative steady-state inactivation properties ($V_{50inact}$ = -93.1 mV) compared to α_{1G} ($V_{50inact}$ = -86.2 mV) and α_{1E} (-81.6 mV). A further distinguishing features of the α_{1I} current is its unusually slow kinetics of activation and inactivation (τ_{act} = 3.1 ms; τ_{inact} = 112.8 ms) compared to α_{1G} (τ_{act} = 0.92 ms; τ_{inact} = 11.6 ms) and α_{1E} (τ_{act} = 1.86 ms; τ_{inact} = 73 ms; all at –20 mV). As is typical for a T-type calcium channel, the deactivation of α_{1I} was slow (τ_{deact} ~ 1 ms at –120 mV) and the permeability for Ca^{2+} was approximately equal to Ba^{2+}. We also tested the effect of several classic T type channels blockers: both α_{1G} and α_{1I} displayed similar low sensitivities to block by Ni^{2+} and weak inhibition by amiloride.

Supported by the MRC of Canada and the Human Frontiers Science Program.

79.6

R.L. Martin[1], M.M. McNulty[1], J.H. Lee[2], E. Perez-Reyes[2], & D.A. Hanck[1]*. [1]Dept. of Medicine, University of Chicago, Chicago, IL 60637 and [2]Dept. of Physiology, Loyola University School of Medicine, Maywood, IL 60153

INACTIVATION KINETICS OF α1I, A LOW VOLTAGE ACTIVATED Ca CHANNEL.

The recently cloned family of low voltage activated Ca channels is comprised of three isoforms (α1G, α1H, and α1I), whose membrane spanning regions share more than 80% homology. When these isoforms are stably expressed in HEK293 cells and studied with 2 mM Ca as the charge carrier, α1I inactivates much more slowly than α1G and α1H. At positive potentials, e.g. at 0 mV, 90% of α1G and α1H current decays by 50 ms, whereas greater than 200 ms is required for 90% decay of α1I. Near threshold potentials (between -80 mV and -60 mV), where inactivation is likely to develop primarily from closed states, availability for all three isoforms reaches steady-state only after 5 s. However, the time courses of both α1G and α1H are bi-exponential, with the slower time constant of ~1 s, accounting for about 20% of the availability. In contrast α1I inactivates with a dominant slow time constant that is near to or greater than 1 s between -80 mV and -60 mV. The presence of such a slow time course of inactivation creates a time dependent window current that would make this channel especially well suited to control excitability in neurons in which it is expressed. Supported by AHA and NIH.

COPY

Atty Docket No.: 381092000721

Inventor: Terrance P. SNUTCH et al.

Application No.: 09/611,257 **Filing Date:** July 6, 2000
Title: MAMMALIAN T-TYPE CALCIUM CHANNELS

Documents Filed:
Transmittal (1 page)

Amendment Under 37 C.F.R. § 1.111 (5 pages)

Copy of the Declaration of Terrence P. Snutch (2 pages)

Via: First Class Mail
Sender's Initials: KHM1/jab15 **Date:** June 27, 2006

Inventor: Terrance P. SNUTCH et al.

Atty Docket No.: 381092000721

Application No.: 09/611,257
Title: MAMMALIAN T-TYPE CALCIUM CHANNELS

Filing Date: July 6, 2000

Documents Filed:
Transmittal (1 page)

Amendment Under 37 C.F.R. § 1.111 (5 pages)

Copy of the Declaration of Terrence P. Snutch (2 pages)

Common Buckeye

MDC

Via: First Class Mail
Sender's Initials: KHM1/jab15

Date: June 27, 2006

PTO/SB/21 (09-04)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	8

Application Number	09/611,257
Filing Date	July 6, 2000
First Named Inventor	Terrance P. SNUTCH
Art Unit	1649
Examiner Name	D. E. KOLKER
Attorney Docket Number	381092000721

ENCLOSURES (*Check all that apply*)

- [ ] Fee Transmittal Form
  - [ ] Fee Attached
- [x] Amendment/Reply (5 pages)
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
  - [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please Identify below):

Copy of the Declaration of Terrence P. Snutch (2 pages)

Return Receipt Postcard

Remarks

Customer No. 25225

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	MORRISON & FOERSTER LLP		
Signature	Kate H. Murashige		
Printed name	Kate H. Murashige		
Date	June 27, 2006	Reg. No.	29,959

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail, in an envelope addressed to: MS Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: June 27, 2006 Signature: Judy Bridgwater (Judy Bridgwater)

sd-323200

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail, in an envelope addressed to: MS Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: June 27, 2006 Signature: Judy Bridgwater (Judy Bridgwater)

Docket No.: 381092000721
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Terrance P. SNUTCH et al.

Application No.: 09/611,257

Filed: July 6, 2000

For: MAMMALIAN T-TYPE CALCIUM CHANNELS

Confirmation No.: 5449

Art Unit: 1649

Examiner: Daniel E. Kolker, Ph.D.

AMENDMENT UNDER 37 C.F.R. § 1.111

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This is in response to an Office Action herein, mailed 14 April 2006, time for response to which was set to expire 14 July 2006. Applicants are deeply appreciative of the withdrawal of many grounds for rejection. The sole substantive issue concerns a publication of the inventors themselves less than one year prior to the application date. Amendments have been made to the claims as suggested by the Office, and a Katz declaration is supplied to remove the cited documents. Reconsideration is respectfully requested.

CLAIM AMENDMENTS

1. (currently amended): [[A]] An isolated recombinant DNA molecule which comprises an expression cassette wherein said expression cassette comprises a nucleotide sequence encoding a T-type calcium channel α_{1G} subunit, said encoding sequence operably linked to control sequences to effect its expression; wherein said α_{1G} subunit has an amino acid sequence identical to SEQ. ID. No.: 24 or has an amino acid sequence identical to SEQ. ID NO. 37.

2. (previously presented): The DNA molecule of claim 1 wherein said α_1 subunit has the amino acid sequence of SEQ. ID NO. 37.

3. (canceled)

4. (previously presented): Recombinant host cells modified to contain the DNA molecule of claim 1.

5. (original): The cells of claim 4 which are mammalian cells.

6. (previously presented): A method to effect production of a recombinant functional calcium channel which method comprises culturing the cells of claim 4 or 5 under conditions wherein said functional calcium channels are produced.

7-13. (canceled)

14. (currently amended): An isolated nucleic acid molecule which comprises a nucleotide sequence encoding a T-type calcium channel α_{1G} subunit or its full-length complement, wherein said α_{1G} subunit has an amino acid sequence identical to SEQ. ID. No.: 24 or has an amino acid sequence identical to SEQ. ID NO. 37.

15-17. (canceled)

18. (previously presented): The isolated nucleic acid molecule of claim 14, wherein said α_{1G} subunit has an amino acid sequence identical to SEQ. ID NO. 37.

19. (previously presented): The DNA molecule of claim 1 wherein said α_1 subunit has an amino acid sequence identical to SEQ ID NO: 24.

20. (previously presented): Recombinant host cells modified to contain the DNA molecule of claim 2.

21. (previously presented): The cells of claim 20 which are mammalian cells.

22. (previously presented): Recombinant host cells modified to contain the DNA molecule of claim 19.

23. (previously presented): The cells of claim 22 which are mammalian cells.

24. (previously presented): A method to effect production of a recombinant functional calcium channel which method comprises culturing the cells of claim 20 or 21 under conditions wherein said functional calcium channels are produced.

25. (previously presented): A method to effect production of a recombinant functional calcium channel which method comprises culturing the cells of claim 22 or 23 under conditions wherein said functional calcium channels are produced.

26. (previously presented): The isolated nucleic acid molecule of claim 14, wherein said α_{1G} subunit has an amino acid sequence identical to SEQ ID NO: 24.

REMARKS

The claims have been amended as suggested by the Office, thus obviating the rejection newly made under 35 U.S.C. § 101 and responding to the objection to claim 14.

Again, applicants greatly appreciate the withdrawal of the rejections previously made.

With respect to the rejection of claims 1, 4-6, 14 and 19-26 as anticipated by McRory, *et al.*, (*Soc. Neurosci. Abstr.* (1999) 25:197) presented at the annual meeting held October 23-28, 1999, this document is cited under 35 U.S.C. § 102(a) and is the work of the present applicants. Applicants note with appreciation that claims 2 and 18, which solely concern SEQ ID NO: 37 are free of this rejection; it is believed that claims 20 and 21, which depend from claim 2 and are therefore restricted to SEQ ID NO: 37, are mistakenly included.

Nevertheless, enclosed herewith is a declaration of Dr. Terrance Snutch verifying that Dr. Snutch's co-authors of the abstract worked entirely under his direction and made no inventive contribution. This declaration should obviate the outstanding basis for rejection over the art. *In re Katz*, 687 F2d 450, 215 USPQ 14 (CCPA 1982) cited at MPEP § 715.01(c).

For purposes of explanation, it is noted that the co-inventor herein, David L. Baillie, does not appear as an author on the abstract. This is because the abstract is an incomplete report of the subject matter described. The full paper describing this work, which appeared subsequent to the filing date herein, is enclosed and includes Dr. Baillie as a co-author. (*J. Biol. Chem.* (2001) 276:3999-4011.)

In view of the amendment to the claims and the enclosed declaration, it is believed that claims 1-2, 4-6, 14 and 18-26 are in a position for allowance and passage of these claims to issue is respectfully requested.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 381092000721.

Respectfully submitted,

Dated: June 27, 2006 By: Kate H. Murashige

Kate H. Murashige
Registration No. 29,959
MORRISON & FOERSTER LLP
12531 High Bluff Drive
Suite 100
San Diego, California 92130-2040
Telephone: (858) 720-5112
Facsimile: (858) 720-5125

Docket No.: 381092000721
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: Terrance P. SNUTCH et al.	
Application No.: 09/611,257	Confirmation No.: 5449
Filed: July 6, 2000	Art Unit: 1649
For: MAMMALIAN T-TYPE CALCIUM CHANNELS	Examiner: Daniel E. Kolker, Ph.D.

DECLARATION OF TERRANCE P. SNUTCH

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Terrance P. Snutch, declare as follows:

1. I am a co-inventor of the claimed subject matter in the above-referenced application. An abstract summarizing some of the work which is encompassed by some of the claims, specifically retrieval of the cDNA encoding a full-length rat α_{1G} calcium ion channel subunit, is reported in this abstract. The individuals listed as co-authors on this abstract, J. E., McRory, J. Mezeyova, C. M. Santi, K. Hamming, and K. Sutton worked with me on this project entirely

under my supervision and direction. They made no inventive contribution to the claimed subject matter.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Executed at Vancouver, British Columbia, Canada, on 21 (day) June (month) 2006.

(Terrance P. Stutch)

UNITED STATES PATENT AND TRADEMARK OFFICE

COPY

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

KHM

MR DOCKETED: Issue Fee

25225 7590 09/21/2006 REMINDER: 11/21/2006

MORRISON & FOERSTER LLP
12531 HIGH BLUFF DRIVE
SUITE 100
SAN DIEGO, CA 92130-20

FINAL DUE DATE: 12/21/2006

DOCKETED: Recon Term Ext.

MR REMINDER:

FINAL DUE DATE: 12/21/2006

SEP 27 2006

EXAMINER	
KOLKER, DANIEL E	
ART UNIT	PAPER NUMBER
1649	

DATE MAILED: 09/21/2006

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/611,257	07/06/2000	Terrance P. Snutch	381092000721	5449

TITLE OF INVENTION: DNA ENCODING MAMMALIAN T-TYPE CALCIUM CHANNELS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	YES	$700	$0	$0	$700	12/21/2006

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

MR DOCKETED: file Div 7

HOW TO REPLY TO THIS NOTICE: REMINDER: 12/21/2006

FINAL DUE DATE:

MR DOCKETED: 312 Amend Option

REMINDER: 11/21/2006

FINAL DUE DATE: 12/21/2006

I. Review the SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

B. If the status above is to be removed, check box 5b on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PTOL-85 (Rev. 07/06) Approved for use through 04/30/2007.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

25225 7590 09/21/2006

MORRISON & FOERSTER LLP
12531 HIGH BLUFF DRIVE
SUITE 100
SAN DIEGO, CA 92130-2040

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/611,257	07/06/2000	Terrance P. Snutch	381092000721	5449

TITLE OF INVENTION: DNA ENCODING MAMMALIAN T-TYPE CALCIUM CHANNELS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	YES	$700	$0	$0	$700	12/21/2006

EXAMINER	ART UNIT	CLASS-SUBCLASS
KOLKER, DANIEL E	1649	435-069100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:
- ☐ Issue Fee
- ☐ Publication Fee (No small entity discount permitted)
- ☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ☐ A check is enclosed.
- ☐ Payment by credit card. Form PTO-2038 is attached.
- ☐ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.
- ☐ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/611,257	07/06/2000	Terrance P. Snutch	381092000721	5449

25225 7590 09/21/2006

MORRISON & FOERSTER LLP
12531 HIGH BLUFF DRIVE
SUITE 100
SAN DIEGO, CA 92130-2040

EXAMINER	
KOLKER, DANIEL E	
ART UNIT	PAPER NUMBER
1649	

DATE MAILED: 09/21/2006

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 0 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 0 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

PTOL-85 (Rev. 07/06) Approved for use through 04/30/2007.

Notice of Allowability	Application No. 09/611,257	Applicant(s) SNUTCH ET AL.	
	Examiner Daniel Kolker	Art Unit 1649	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *6/30/06*.

2. ☒ The allowed claim(s) is/are *1,2,20,21,24,19,22,23,25,4-6,14,18,26, renumbered as 1-15 respectively*.

3. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

4. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☒ Information Disclosure Statements (PTO-1449 or PTO/SB/08), Paper No./Mail Date 7/19/06
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application (PTO-152)
6. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
7. ☒ Examiner's Amendment/Comment
8. ☐ Examiner's Statement of Reasons for Allowance
9. ☐ Other _____.

ROBERT C. HAYES, PH.D.
PRIMARY EXAMINER

DETAILED ACTION

1. Applicant's remarks, amendments, and declaration filed 30 June 2006 have been entered.

2. All objections and rejections are withdrawn in light of the amendments and declaration.

3. An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

The application has been amended as follows:

On the first page of the specification, the title has been amended as follows:

- - DNA Encoding Mammalian T-Type Calcium Channels - - -

Conclusion

4. Claims 1 – 2, 4 – 6, 14, and 18 – 26 are allowed.

5. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Daniel Kolker whose telephone number is (571) 272-3181. The examiner can normally be reached on Mon - Fri 8:30AM - 5:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Janet Andres can be reached on (571) 272-0867. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Daniel E. Kolker, Ph.D.
September 12, 2006

ROBERT C. HAYES, PH.D.
PRIMARY EXAMINER

ALTERNATIVE TO PTO/SB/08a/b (07-05)

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete If Known	
Application Number	09/611,257
Filing Date	July 6, 2000
First Named Inventor	Terrance P. SNUTCH
Art Unit	1649
Examiner Name	D. Kolker
Attorney Docket Number	381092000721

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]

*EXAMINER: Initial if information considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST. 16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
DK	1.	McRORY et al., Journal of Biological Chemistry (2001) 276(6):3999-4011	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1]Applicant's unique citation designation number (optional). [2]Applicant is to place a check mark here if English language Translation is attached.

Examiner Signature	/Daniel Kolker/ (08/24/2006)	Date Considered	08/24/2006

sd-325091

Currently Pending Claims

28. A method to identify an antagonist of a T-type calcium channel which method comprises:

a) contacting a recombinant cell expressing the α_1 subunit of a heterologous T-type calcium channel with a known agonist of said T-type calcium channel;

b) contacting said cell with a compound to be tested; and

c) determining the ability of said compound to diminish the activation of said α_1 subunit by said agonist;

wherein said α_1 subunit is functional as a T-type calcium ion channel and is encoded by a nucleotide sequence which hybridizes under conditions of stringency corresponding to washing at 62° C in 0.2 x SSPE/0.1% SDS to a nucleic acid comprising SEQ ID NO: 23 and

wherein said activating comprises enhancing the flow of calcium ions into said cell in the presence as compared to the absence of said agonist;

whereby a compound which diminishes the activation of said α_1 subunit by said agonist is identified as an antagonist.

29. The method of claim 28 wherein said activation is measured by measuring the current through the calcium channel before and after said contacting of said cell with said compound.

30. The method of claim 28, wherein said cells contain a fluorescent dye sensitive to intracellular calcium concentration and said activation is determined by observing a change in the fluorescence of said dye when said contacting is performed.

31. A method to prescreen compounds as agonists or antagonists of T-type calcium ion channels by virtue of their ability to bind said T-type channels which method comprises:

a) contacting a recombinant cell expressing the α_1 subunit of a heterologous T-type calcium channel with a compound to be tested; and

b) determining the ability of said compound to bind to said cell expressing said α_1 subunit;

wherein said binding is determined by observing competitive binding with a known agonist or antagonist of said channel;

wherein said α_1 subunit is functional as a T-type calcium ion channel and is encoded by a nucleotide sequence which hybridizes under conditions of stringency corresponding to washing at 62°C in 0.2 x SSPE/0.1% SDS to a nucleic acid comprising SEQ ID NO: 23,

whereby a compound which is determined to bind said cell is identified as a compound which will behave as either an agonist or antagonist of a T-type calcium channel.

37. The method defined in claim 28 wherein the nucleic acid comprises SEQ ID NO: 23.

40. The method defined in claim 31 wherein the nucleic acid comprises SEQ ID NO: 23.

PTO/SB/21 (09-04)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number Appeal Number	09/346,794 2006-2389
Filing Date	July 2, 1999
First Named Inventor	Terry P. SNUTCH
Art Unit	1646
Examiner Name	N. S. Basi
Attorney Docket Number	381092000720

Total Number of Pages in This Submission	64

ENCLOSURES (*Check all that apply*)

- [ ] Fee Transmittal Form
  - [ ] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
  - [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please Identify below):
  1) Reference to Related Case and Verification of Status (3 pgs)
  2) Copy of Notice of Appeal (6 pgs)
  3) Copy of Request for Review (4 pgs)
  4) Copy of Notice of Panel Decision (2 pgs)
  5) Copy of Office Action (30 pgs)
  6) Copy of Response to Office Action (9 pgs)
  7) Copy of Notice of Allowance (7 pgs)
  8) Copy of Pending Claims (2 pgs)
  9) Return Receipt Postcard

Remarks

Customer No. 25225

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	MORRISON & FOERSTER LLP		
Signature	Kate H. Murashige		
Printed name	Kate H. Murashige		
Date	October 2, 2006	Reg. No.	29,959

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EV 915685902 US, on the date shown below in an envelope addressed to:MS Board of Patent Appeals and Interferences, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: October 2, 2006 Signature: Judy Bridgwater (Judy Bridgwater)